

Handling the human embryo

Proposals for the organization of British practice are a useful curtain-raiser for the government's own report. But physicians should regulate themselves before the government steps in.

THE Council for Science and Society has usefully stolen a march on the British Government by publishing (last week) the report of a study of new techniques of conception, some old (such as artificial insemination), some futuristic (such as cloning). The document (*Human Procreation*, OUP, £3.95) has been put together by a committee under Dr G.R. Dunstan, an academic Anglican with at least fifteen years' experience of these problems, but with a good sprinkling of hard and level-headed biologists to help tell one end of a blastocyst from another. The document appears some three months before the report of the official committee under Mrs Mary Warnock is due to report. Being a liberal and even modestly adventurous tract — Dunstan would allow the use of fertilized but unimplanted embryos for research, is neutral on surrogate motherhood (saying that it should neither be encouraged nor made unlawful) and sternly dismissive on the choice of the gender of unborn children. In all probability, this is where the Warnock committee will stand. Certainly that committee's recommendations on the legal framework for embryonic manipulation cannot be less liberal without seeming unhelpful.

The Dunstan document is said to have been designed to be thought-provoking, which mostly it is. But on some issues the group seems to have decided that a dash of pragmatism is the only sensible way of avoiding arguments that can never be settled. Thus the committee dodges the question of when life can be said to have "begun" by argument by analogy with the law on abortion in the United Kingdom. Sperms and ova are alive, so "life" must mean something else, but development is continuous after fertilization so why not extend the limiting age of a fetus for legal abortions to other practices now possible for the first time, the study of development in viable embryos, for example? The trouble, as the Dunstan committee must know, is that there is a substantial body of opinion in Britain opposed to that limit, and eager to see the Abortion Act repealed. If the Warnock committee makes recommendations along the same lines (which it should), there is certain to be a revival of agitation against the rules on abortion.

Privacy is dealt with more robustly. The issue arises in connection with existing practices such as artificial insemination (estimated at 4,000 cases a year, not much greater than in the early 1970s) but, more generally, whenever a child is born with genes

not all derived from its social parents. On the question whether such a child should at some stage be told something of the source of the genes, the Dunstan committee is for "openness within the family", which would imply that a child would eventually have a right to know as much about, say, the donor of semen as the parents themselves were told at the outset. But the committee would protect the confidentiality of the donor, largely because it fears that the supply of donors would otherwise dry up but also because it holds that a child's relationship with its social parents should not be prejudiced by the intervention of a third party, either a semen donor or a surrogate mother. The view is consistent. The obvious difficulty is that responsibility for keeping genetic records of these shadowy but essential third parties will rest with physicians whose practices are already shadowy enough. But especially in Britain, where the Data Protection Bill will do very little to strengthen the privacy of individuals in relation to the state, it would be too dangerous to ask for a centralized data bank of the genetic parents of other people's children. Is there a case for physicians' professional associations shouldering this task?

The committee's weakest conclusion is that it would not be permissible to allow parents to choose the gender of their children. Technically, this can already be done by amniocentesis and abortion, but an unwelcome gender is not a legal ground for abortion. Soon, easier ways of doing this job will be available. The starting point for much of Dunstan's argument is that, if stable couples are prevented by some disability from procreating naturally, they should be helped by whatever techniques are available to produce children in some other way. Why draw the line at gender-rigging? A farming family of a dozen daughters and no sons may be as seriously handicapped as one that is infertile. So why not acknowledge that physicians should be encouraged to offer even such a service if there are strong grounds for believing that the results would be socially beneficial?

With its pragmatic view of the problems of embryo manipulation, the Dunstan committee inevitably is less concerned with the legal than the administrative changes of the framework in which the physicians deal with their patients. (But it would change the law to protect donors of sperm from paternity suits.) Writing for a British audience, it asks that the National Health Service should take responsibility for providing whatever services are available, that donors (of anything) should not be paid for their services and that there should be more provision for counselling than at present. The committee looks enviously across the English Channel at the centralized service by means of which the French health services arrange for sperm donors to be married couples with at least one healthy child. The committee acknowledges that there are problems with surrogate mothers (who would not act as hostesses without reward of any kind) but seriously underestimates the difficulties of ensuring that all services like these can somehow be swept up into the public sector. That is why there can in the long run be no escape from a system in which individual practitioners offering services as different as artificial insemination and sex determination should be registered (even licensed) by their professional associations and should contribute data about their practice to some confidential data bank run by the same association. That, it is to be hoped, is what Warnock will say. □

Liberation language

THE introduction to the Dunstan report by Professor John Ziman, who quotes from Dante's *Purgatorio* a definition of life as

... one

quick, sentient soul, of its own self-aware,

includes the following explanation of a now-unfashionable usage:

The report is written in English, a language which has a common gender for statements applying to masculine and feminine, male and female, without distinction. Sometimes the grammatical form of the common gender and masculine coincide, but that is no impediment to its use. We have employed this grammatical facility because it yields a prose less cluttered and more elegant, and as readable. □



Small beer, no skittles

The British research system needs to spend more on research grants, less on machines.

UNWITTINGLY, the British Science and Engineering Research Council (SERC) has given a telling explanation of why the United Kingdom is considering withdrawal from the European high-energy physics laboratory, CERN, at Geneva. The council's science board, the chief source of research grants for British academics whose interests are not bound up with space, astronomy and high-energy physics, last week published a statement of what it calls its strategy for "core science" — what is left when the great machine-created baronies have been catered for (see *Nature* 309, 297; 1984).

The results are shocking. The board spends just over £70 million a year, which is divided into three roughly equal parts, one of which goes to keep the bodies and souls of graduate students and doctoral fellows in some loose correspondence, another on the running of central facilities (such as synchrotron and laser sources) and a third, roughly £25 million a year, on research grants to working scientists in all disciplines. Having proudly calculated that 12,000 people are dependent on it for research money, it omits the simple arithmetical calculation that there is less than £2,000 a year for each person on its books.

Lopsidedness also leaps out from this eight-page glossy production. University research in physics, for example, costs the board between £5 and £6 million a year. This is hardly the kind of cash flow on which a strategy could be founded. The board says that it is having to refuse nearly a third of grant applications that deserve to succeed. Maybe it should be worrying instead that, with such tiny sums of money at its disposal, it is not much more seriously overwhelmed.

Balkanization

To be fair to all concerned, these sums are not the whole of what the British research councils spend on the support of academic research. The specialized spending boards into which SERC is balkanized also make grants to academics but, of necessity, the successful applicants there are almost always those whose projects dovetail neatly with the planned use of some machine, observatory, Earth satellite or the like. Other research councils chip in as well; the Medical Research Council spends more on academic biological research than does the science board by a factor of two; the Agricultural and Food Research Council, more deeply committed to its own institutes, also helps, as does even the Natural Environment Research Council. The result, however, is a conspicuous lopsidedness. Why are physics and chemistry research, core sciences if there are any, supported on such a miserly scale?

The usual answer is that that is how the pattern of spending has developed, and that the supply of research grants cannot be too different from the pattern of demand, because the mechanism is self-correcting. This complacent argument overlooks the simple truth that if you starve a field of funds, and spend the best part of a decade telling academic scientists that they would be better employed in other fields, in information technology or engineering for example, they will eventually go elsewhere, intellectually or geographically.

Core science

In Britain, the problems of core science have been multiplied by the perverse pursuit of over-rational stratagems for economizing in resources. High-powered lasers are in fashion? Then the managers of the research enterprise quickly calculate that they could not provide every likely group with up-to-date equipment so let there be a single central facility that everybody can use, well designed (and slowly built). This is probably the most effective device yet discovered for turning honest scientists into committee-people, skilled above all at winning allocations of time at facilities which are always more elaborate than would have been necessary for a single purpose — and whose lifetime is assured for years and even decades to come. So it turns out that with the passage of

time, young men who embark with enthusiasm on working as attendants of the big machines (even the small big machines) discover that they are no longer practitioners of the craft that attracted them but rather managers, negotiators, sometimes even diplomats.

Nobody suggests that it would be possible to turn back the clock so far that the practice of science in the physical sciences would be exclusively modelled on what happened in the nineteenth century. It is however likely, with the present distribution of resources in Britain between machine science and small-scale science, that the pendulum has swung far too far towards the machines. There is no evidence that people dislike working with the large machines but that is not the point. What matters is that the way in which the machines claim people's allegiance for years on end ensures that the community of scientists in a country as small as Britain is less flexible than it might be, less able to seize new opportunities.

The science board seems at least to have understood the way in which changes should be brought about. Its strategy document says that it needs more money and that if it had more money it would give first priority to research grants and the last to the strengthening of machine facilities. But how long does the science board think it would take to effect a noticeable change in the pattern of scientific research if on the one hand it is going to adjust its distribution of resources only when the total is increasing and if at the same time it follows its own prescription of giving particular attention to projects which have industrial potential? One of the constantly repeated mistakes in the management of British civil science in the past few decades is that with each new economic crisis, the research councils whose responsibility is to ensure the health of basic science have felt it necessary to demonstrate that they too have to put their shoulders to the wheel, helping with chemical engineering one decade, information technology the next.

Mercifully, there are signs that even this preoccupation may soon be modified. In the past six months, SERC, recognizing the dangers of over-dependence on machines, has tried to create a set of circumstances in which the two big spenders among its committees, the astronomy, space and radio board and the nuclear physics board, would be merged, presumably so that they would then take a more sceptical view of what their enforced partners were supporting. It is a well-known failing of even the best intentioned systems for distributing money for research that peer review is harder to trust as the scale of projects grows. Unfortunately, SERC has for the time being defeated its chairman's plan to bring about this marriage. He (or his successor) should not however let the matter rest there. The case for having rational means of distinguishing between the merits of different proposals is too strong to be forgotten.

The grant-making establishment needs also to safeguard itself against repetition of the mistakes that have recently caused such trouble. Logical though it is to think of serving many users and saving money at the same time by the provision of central facilities which all experimenters can use, the system is also a recipe for diseconomy. Would it not be better, as in the United States, that if a person thinks it would be valuable to have a machine for accelerating particles, or producing intense monochromatic beams of light, or even perhaps a machine for producing neutrinos, he should not be prevented from applying for the money he needs by the argument that the project is too big for one man to handle? Of course that is literally true. But elsewhere, in the United States particularly, there is now a long record of successful exploitation of the principle that the most enthusiastic user of a novel kind of machine is likely to be the man who gave the reasons why it should be built. And then, of course, if two people have the same idea for a new machine at the same time, there can be a competition. Either way, the enterprise is likely to be carried out more economically than would otherwise be possible, fewer people will acquire a vested interest in its perpetual continuation and, indeed, there would be many occasions when designers of new experiments on a large scale were treading on their own heels, anxious to get rid of what they had been anxious to build only a little while earlier. □

American Association for the Advancement of Science — New York

Administration hints at clampdown on biotechnology exports

AMID charges from the Department of Defense (DoD) and US intelligence agencies that the Soviet Union is applying biotechnology to biological warfare research, the Reagan Administration is beginning to suggest that biotechnology may have to be brought under export control regulations designed to halt the flow of "militarily critical technologies" to potential adversaries.

The Reagan Administration's interpretation of the export control rules has been the subject of controversy, particularly in the crackdown on exports of computer components and in DoD's recent efforts to shut out foreign nationals — even those from NATO countries — from scientific meetings held under its sponsorship.

At the AAAS meeting here, John Birkner of the Defense Intelligence Agency predicted that "dual use" biotechnology — items with both peaceful and military applications — would sooner or later have to be restricted. He asked scientists in industry and at universities to help DoD learn "how our technology may be turned against us" so that a "prudent" list of militarily critical technologies can be compiled for biotechnology.

Items listed as militarily critical can be exported only with a licence issued by the Department of Commerce. To qualify for the list, a technology must not already be possessed by "principal adversaries" nor available to them from a third party. Birkner said that specialized polymers, synthetic elastomers and detectors for chemical and biological warfare agents would all be likely candidates for inclusion on the list.

The charges that the Soviet Union is already exploiting biotechnology for biological warfare have emerged in recent weeks from official DoD sources, the Central Intelligence Agency and a series of articles in the *Wall Street Journal*. DoD's recent report on Soviet military power asserts that the Soviets have an active research and development programme to investigate the utility of biological weapons; Birkner said that "there are at least seven biological warfare centres in the Soviet Union that are under strict military control" and which are applying "selected aspects of genetic engineering to their work". The *Wall Street Journal* articles made similar accusations, and in turn have been sharply criticized by many US researchers for imputing sinister motives to research activities that are indistinguishable from US efforts in biotechnology and basic genetic engineering.

The putative activities of the Soviet Union would constitute a violation of the

1975 convention banning the possession or development of biological weapons. The United States had earlier accused the Soviets of violating the treaty (and the 1925 chemical warfare treaty) by using toxin and chemical weapons in Afghanistan and South-East Asia.

Meanwhile, some new light has been shed on the US military's own programme of recombinant DNA and hybridoma research. Thomas Dashiell of the Office of the Secretary of Defense appeared at the meeting here with a list of 43 biotechnology research projects funded by DoD as of 30 April. The bulk of the projects deal with development of vaccines against disease endemic in areas where US troops might be sent or against possible biological warfare agents, such as anthrax. Other projects are aimed at developing enzymes to decontaminate chemical warfare agents and at producing materials such as epoxies, lubricant stabilizers, biofuels and marine anti-fouling agents.

The research, which is divided between in-house efforts (mainly at Fort Detrick in Frederick, Maryland — the former biological warfare research establishment) and sponsored research at universities (among them Massachusetts Institute of Technology, Texas A&M University, Rockefeller University, Purdue University and the Universities of California, Massachusetts, and Maryland), is all unclassified. Dashiell suggested, however, that if the research moves beyond the basic stage — which he says is not likely to happen for eight years at least — some of the development work may be subject to secrecy restrictions.

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Peter David adds: In Washington meanwhile, Congress received an unexpected signal last week that the Reagan Administration may be softening its stance on the control and publication of research findings. Dr Edith Martin, Deputy Under-Secretary of Defense for research and engineering, announced during a hearing of the House Science and Technology Committee that the administration intended to rely on formal classification procedures to keep sensitive research out of the hands of foreign nations.

Apparently reading from the still-unpublished report of a senior interagency group examining technology transfer, Dr Martin said it was the policy of the administration that "no restrictions may be placed on the conduct or reporting of research that has not received national security classification".

Although the statement referred specifically to "fundamental" research, it was taken by observers at the AAAS meeting to

imply that the Department of Defense (DoD) had dropped a controversial plan to restrict the publication of applied research that was sensitive but not formally classified. Drafted as part of a wide-ranging review of the Pentagon's approach to international technology transfer, the plan would have insisted that DoD contracts for work that was both "applied" and "sensitive" should in future give the Pentagon the right to prevent publication of the resulting findings.

In meetings with universities in recent months, Dr Martin had argued that total reliance on classification, in which it would be necessary to show that publication of the research would constitute a direct threat to national security, was too inflexible. But her proposal to create a new category of sensitive research whose publication would be controlled by DoD was fiercely opposed. Three universities — Stanford and the California and Massachusetts Institutes of Technology — wrote to DoD and to the White House science office claiming it would prevent them from undertaking certain categories of research for DoD. □

Europe uneasy at CERN rival

Dr Herwig Schopper, director general of the European Organization for Nuclear Research (CERN), is sowing seeds of dissent to the ambitious US plan for a massive new particle accelerator. Speaking here at the AAAS meeting, Schopper suggested that the tunnel now being completed for LEP, CERN's 100-GeV electron positron collider, could be used for a future proton machine with capabilities approaching those of the US design, and at substantially lower cost.

While careful to stress the importance of "complementarity" between European and US efforts in high-energy physics, Schopper clearly implied that the United States could find itself competing with CERN for the claim to the next large accelerator.

The United States made an initial commitment to its proton collider, known formally as the Superconducting Super Collider (SSC) (and earlier known as the "Desertron"), last year when the High Energy Physics Advisory Panel endorsed the concept. The panel, which represents the particle physics research community and which makes recommendations to the Department of Energy (DoE), at the same time voted to abandon Brookhaven ►

National Laboratory's unfinished ISABELLE accelerator, recognizing the unprecedented concentration of technical and financial support that SSC would require.

As now envisaged, SSC would attain centre-of-mass energies in the neighbourhood of 40 TeV — sufficient, in the view of current theory, to explore the so-called Higgs sector. Leon Lederman, director of Fermilab, who also spoke here, said that experimental exploration of this energy region is vital to resolving the "crisis" that Lederman says his "theoretical colleagues" now find themselves in. "They're speculating wildly", he says, for lack of solid data.

According to cost estimates developed earlier this year at a marathon session at Lawrence Berkeley Laboratory (some 150 researchers from the national laboratories and universities were, as one participant put it, locked in a room by Department of Energy officials until they could agree on some definite numbers to be used in preparing next year's budget request), SSC could be built using existing magnet technologies for \$3,000 million, with a completion date in 1994. DoE is now considering whether to submit a request for research and development funds in the budget for fiscal year 1986. Lederman says that it would probably take two or three years to develop a definite design that optimizes costs. The "reference design" document produced at Lawrence Berkeley considered three different combinations of ring size and magnet field strength, and concluded that costs would be roughly equal in each case. The original idea of SSC, and thus the name Desertron, was to use relatively low field, "cheap" magnets in an enormous ring that could presumably fit nowhere but the southwestern desert.

Lederman said he considered the \$3,000 million figure to be a maximum, as it assumes the use of existing "dumb" magnet technology, as he put it.

Schopper, on the other hand, placed a price tag of \$500 million on the European version of the proton collider, the cost savings coming from the use of CERN's existing tunnel and injectors. The energy of the machine, however, would be only 10 to 12 TeV if existing magnets technology is assumed, although it could be as much as 18 TeV if the as yet undeveloped Nb₃N 10-tesla magnets become available in time.

Both Lederman and Schopper spoke of the need to coordinate US and European plans; both also said they had little idea how this could actually be accomplished. Lederman said, however, that it would probably be a political impossibility to persuade the countries in question to build both machines. He also questioned whether the CERN proton collider's energies would be sufficient to explore the Higgs sector.

Schopper, in the face of repeated questions about the relative cost-effectiveness of the two plans, said "It's no use starting a

fight now"; more work needs to be done on the technology before a decision can be made, he said. And, apparently referring to hints of some new and puzzling discoveries coming out of CERN's SPS collider, suggested that in the two to three years needed for technology development, "the physics might be different".

Schopper suggested that another possible add-on to the LEP tunnel — one that certainly would not compete with SSC — would be an electron-proton collider.

Intellectual property

Consider the monoclonals

BRITISH universities seem to be rebelling against the mounting pressure on them to become more responsive to commercial considerations. The Committee of Vice-chancellors and Principals (CVCP), in a preliminary response to a government discussion paper on intellectual property rights, rejects the idea that universities should seek to protect research inventions with patents wherever possible, citing as an example the "serious disadvantages for generations of investigators" if monoclonal antibodies had been rigorously protected. And in a formal reply to a plea by the Engineering Council to spend more of their grant on training engineers, the committee reaffirms its opposition to having grants specially earmarked for engineering and its distaste for a rapid increase in provision for science and technology at the expense of other subjects.

CVCP dismisses the government's "green paper" on intellectual property, published at the end of last year, as "somewhat superficial". The paper made some radical suggestions on how the Patent Office might be moved out of the civil service and suggested some changes to intellectual property law. But, say vice-chancellors, it pays too little regard to the difficult balance that university researchers must maintain between possibilities for commercial exploitation and their commitment to intellectual progress. And CVCP dismisses the "implied criticism" that academics exchange information too freely and take protection too rarely. Universities have, the committee says, a clear policy on patenting. The government should, before considering legislation, thoroughly examine all aspects of intellectual property, including such problem areas as copyright and computer software. Some are concerned that biotechnology, in particular, might be adversely affected if very broad patents impede research.

Beyond this CVCP does not go, thus perhaps inviting the criticism that it, too, is being somewhat superficial. The committee excuses itself by blaming an unrealistic timetable for comment imposed by the Department of Education and Science. Discussion has had to be brief and consultation has been less than complete. A

Meanwhile, the United States is already worrying about selling the most expensive basic research project yet to Congress. Presidential science adviser George Keyworth was recently in Japan trying to drum up some tangible support from the Japanese Government for the project. And DoE officials are only half facetious when they suggest that the SSC ring might be arranged to pass through at least three states to boost regional support in Congress.

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fuller response will be prepared in due course.

The committee has however managed to find the time to prepare a full response to the Engineering Council, which earlier this year accused universities of not using their grant in the way that was intended. The council announced that it wanted a ten per cent swing in student numbers from arts-based to science-based subjects within the decade. That objective is thought by vice-chancellors to be totally unrealistic, given the pressures that already exist on the university system, and many vice-chancellors feel the Engineering Council has weakened its case by overstating it. **Tim Beardsley**

Argentines safe

Washington

THE new democratic government in Argentina is wasting no time in trying to re-establish contacts with expatriate Argentinian scientists and to offer assurances that the personal and political vendettas of past regimes will not be repeated.

Dr Carlos Abeledo, under-secretary for science promotion and director of the national research council of Argentina, was in the United States last week to spread the word among the hundreds of Argentinian scientists here that if they want to return they will be welcome and there will be financial support for them. Abeledo said that he did not expect more than a few dozen to take up the offer this year, but that he was also hoping to involve the expatriate scientists in the country's scientific effort through such activities as reviewing for Argentinian scientific journals, reviewing grant applications, and taking in Argentinian research fellows.

Abeledo was due to meet with some 200 Argentinian scientists at the American Association for the Advancement of Science meeting in New York; the previous week he was present at a gathering of 100 Argentinian scientists now living in France.

Abeledo was among the hundred or so scientists sacked in 1976–77 for political — and sometimes personal — reasons by the government. One of the first acts of the new government this year was to recognize the right of those scientists to return to their positions.

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US universities

Benefactions abound

Washington

UNIVERSITIES in the United States are enjoying a spectacularly profitable spring this year. In April alone, the University of Texas at Austin announced that an \$8 million donation by an anonymous Texan would enable it to endow 32 new chairs in science and engineering, and the University of California said that it had received the biggest single private gift in its history: \$36 million for a new telescope. There may be more to come. According to a new survey, universities and colleges are becoming America's favourite philanthropy: voluntary contributions to higher education reached a staggering \$5,000 million in 1982-83, an increase of 6 per cent over the previous year.

The University of California gift comes in the form of cash, property and art masterpieces bequeathed by Mrs Marion Hoffman, widow of the car dealer, Max Hoffman. The money is to form part of a \$100 million fund with which the university intends to build a 10-metre telescope of novel design at Mauna Kea on Hawaii by the end of the decade. It will consist of 36 six-foot hexagonal mirrors arrayed to form a continuous optical surface. The mirrors will be kept aligned to a precise optical figure by a system of computer-controlled sensors and actuators capable of performing 100 positional readjustments a second.



It will have four times the light-gathering power of the venerable Hale Telescope on Mount Palomar.

A bonanza of similar proportions will befall the University of Texas as a result of the \$8 million donation by a local industrialist. The \$8 million is to be matched by five Texan foundations, and another \$16 million will come from the state-funded Permanent University Fund. Together, the package will enable the university to establish 32 chairs endowed for \$1 million each. The university expects filling the chairs to take several years. It will be recruiting in chemistry, physics, mathematics, molecular biology, computer engineering, microelectronics, computer-assisted design and manufacturing and

materials engineering.

The universities of California and Texas are traditionally among the biggest recipients of private and corporate philanthropy. A survey now published by the Council for Financial Aid to Education reports that, in 1982-83, voluntary donations to the University of Texas exceeded \$107 million, while the University of California received more than \$135 million. In proportion to their size, however, neither matches Harvard and Stanford, which received donations of more than \$126 million and \$91 million respectively.

Where is all this money coming from? Increasingly, it seems, from private business. Corporate philanthropy has at last outstripped donations from foundations and reached more than \$1,000 million in 1982-83, 14 per cent more than in

the previous year. That means that the lion's share of donations comes from four distinct sources: corporations, foundations, alumni and private individuals. Each gives about \$1,000 million in a year, while religious denominations give an estimated \$200 million in total.

Nearly a third (\$1,500 million) of all the money contributed is in the form of unrestricted gifts. Some \$300 million is earmarked for academic salaries and \$750 million for research. But the gifts are distributed extremely unevenly. Major private universities receive an average of \$21 million each, about five times as much as their public counterparts. And ten universities accounted for a full \$690 million of the gifts in 1982-83. Harvard and Stanford lead the pack. Eight other institutions, receiving between \$50 million and \$60 million each, are: Minnesota, Columbia, Cornell, Massachusetts Institute of Technology, Yale, Princeton, Southern California and Pennsylvania.

Peter David

Genetic engineering

Picking up the pieces

Washington

PROPOSALS from private companies for the field-testing of recombinant organisms will still be considered by the Recombinant DNA Advisory Committee (RAC) of the US National Institutes of Health (NIH), despite the cloud created by a federal district court ruling in favour of anti-genetic-engineering activist Jeremy Rifkin (see *Nature* 24 May, p.296). That ruling has temporarily halted Dr Steven Lindow's experiment on ice-nucleating bacteria and barred RAC from approving any other experiments involving NIH funds that would deliberately release recombinant DNA into the environment.

But the ruling specifically exempted private companies, which are not legally bound by RAC decisions, although they have been complying on a voluntary basis. The reasoning was that if RAC was not allowed to consider proposals from private companies, the companies could simply proceed without RAC's approval.

Although a final decision has not been made by NIH, RAC staff said last week that as far as they knew two deliberate release proposals from private companies were still on the agenda for the next RAC meeting, on 1 June. Advanced Genetic Sciences Inc. (AGS) is seeking approval for a field trial virtually identical to Lindow's, and Cetus Madison is submitting a proposal for the field testing of plants with genetically-engineered disease resistance. RAC will hear all of the Cetus Madison proposal and part of the AGS proposal in closed session, in accordance with the standard procedures for the safeguarding of trade secrets.

In a letter to the executive director of RAC dated 18 May, Rifkin requested a

moratorium on consideration of all deliberate-release proposals and said he was appealing the district court's decision on that point. Rifkin's attorney, however, said they would not appeal until RAC had decided definitely on how to handle the June meeting.

Meanwhile, the University of California has filed an emergency appeal against the temporary injunction against Lindow's experiment. According to Lindow, the experiment must begin by around 25 May or be postponed until autumn. William Anderson, attorney for the university, said that the court failed to examine RAC's deliberations on the Lindow proposal, which, he said, met the legal requirements of a "hard look" at environmental effects. The appeal also argues that Rifkin failed to exhaust available administrative remedies before filing suit, as he failed to file comments or attend either of the RAC meetings at which the Lindow proposal was discussed. Rifkin says he did not know about those meetings and was out of town when they took place; announcements of the meetings and requests for public comment were, however, published in the *Federal Register*.

Rifkin's attorney, Edward Lee Rogers, said that he thought neither argument would stand up; the *Federal Register* announcement was "cryptic", he said, and did not make clear that this was the first deliberate-release proposal; and he said the "equivalency" defence — that RAC in fact carried out the equivalent of a legally-required environmental assessment — is available only to agencies whose principal responsibility — and thus expertise — is with environmental issues.

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Venture capital

Stealing universities' thunder?

A NOVEL Anglo-US joint venture launched this week aims to sponsor the commercial development of research projects carried out in British universities. The two partners in the new venture, to be called the Research Corporation Trust, are Investors in Industry (3i) and the Research Corporation, of New York. The trust will, through a subsidiary, evaluate, patent and license inventions throughout their lifetimes and will use the proceeds to sponsor more university-based research.

The trust is likely to prove controversial. In return for its free advisory and patenting service to universities the trust will normally expect resulting patents to be made over to it. The obvious question is whether the fruits of research supported by the British taxpayer might not be enjoyed overseas. Mr Ivan Momtchiloff, a general manager of 3i and a director of Research Corporation Trust, said recently that British inventions would be exploited by the trust in Britain "where humanly possible", unless to do so was "obviously silly". A slightly different line has been taken by Dr John P. Schaefer, president of Research Corporation, who says the trust "will not only help strengthen science and technology in the United Kingdom but also lead to a mutually beneficial exchange of technology between the United States and Europe."

The two partners in the new trust are not obvious bedfellows. Research Corporation in the United States is incorporated under not-for-profit law, and the new trust in Britain will, it is hoped, receive charitable status. The Investors in Industry group is, however, an established large industrial investment group, backed by major British banks, whose goal is undeniably to make a profit. Mr Momtchiloff says that 3i will profit from any companies that it acquires or spawns to develop the inventions originated by the trust, and from licence income.

A major spur to the formation of the new trust has been the recent abolition of the first-refusal right on much publicly-funded British research previously enjoyed by the National Research Development Corporation (now part of the British Technology Group). The 3i group has previously formed companies based on university research but the process has often been protracted and difficult. The group was, according to Momtchiloff, considering a new approach based on research grants when it learnt that Research Corporation in the United States was thinking of setting up a British operation; a partnership was the natural result. The corporation already has the special skills and extensive experience of working with university departments to enable a similar scheme to be set up in Britain. The trust will also benefit from Research Corporation's established patenting and legal support services.

The initial capital in the new scheme is

surprisingly modest — £1 million from each of the two partners, with an additional £50,000 each to start the ball of research grants and license income rolling. Many of the initial grants will be for only a few thousand pounds — to buy a specific item of equipment for instance. The trust will seek mainly to interest young researchers who may have more difficulty than their elders in attracting research funds.

The trust's share of royalty income, net of expenditure, will be returned from the subsidiary operating company to be distributed as more research grants. In the United States the arrangement between universities and the new trust's parent company is that the institutions take 60 per cent of royalties and the trust 40 per cent. Arrangements between institutions and their employees are more variable, and the opportunity to take an equity stake in a new company would depend on the individual circumstances.

Tim Beardsley

A UK venture

A VENTURE capital fund launched last week in Britain will provide £15 million over 4 years to invest in new and emerging private companies working in high technology. The Charterhouse Japhet Venture Fund has already raised this sum and has made its first investments. Its preferred areas are telecommunications, microelectronics, health care and biosciences.

The capital that the new fund has available puts it immediately in the ranks of the larger venture funds in Britain. The investors are prominent UK pension funds. The investment directors are Dr John Walker, who comes from the Investors in Industry group, and Mr Ron Sheldon. Walker emphasizes that his approach extends to providing active management support and marketing experience.

Most of the investments will be of more than £250,000, but some "seed capital" for new product development will also be available. The £15 million will spread over 20 to 25 major investments, of which the fund will be the lead investor in about a half. Walker is critical of other venture capital funds that attempt to gain control over a young company by taking a majority investment. He swears that the new Charterhouse fund will always take a minority stake so that control remains with the originator of the company, a principle Walker considers extremely important.

Walker has a significant personal stake in the new fund. He declines to reveal the amount but admits that he "probably won't have to worry about cash" if the fund succeeds. He and Sheldon, by demonstrating their personal commitment, will inspire company management to confidence, he believes.

Tim Beardsley

Agriculture

Old crop ripe for revival

Waalre, The Netherlands

ONE of the first plants to be exploited by man in South-East Asia and Oceania could once again become a major commercial crop in the region if the efforts of Professor Michiel Flach of the Agricultural University at Wageningen bear fruit.

The crop is the sago palm, natural stands of which cover some 2 million hectares in Indonesia, Papua New Guinea and the Philippines. These areas are under-exploited, says Professor Flach, in a region where it would be prohibitively expensive to plant other crops. There are also 200,000 hectares of planted sago stands, mostly in Malaysia. There is a small but growing market for sago starch, not only for food use and industry (textiles and paper) but also for production of high-fructose syrup.

Professor Flach's espousal of the sago cause began in 1970, and with backing from the World Bank, the Food and Agriculture Organization (FAO) and the Dutch Government he organized international symposia on the subject in 1977 and 1980. Earlier this year there was a discussion on sago at an FAO workshop in Jakarta, Indonesia and Flach is now organizing a third symposium — this time in Japan where there is considerable interest in the crop.

A new sago starch factory is to be constructed in Indonesia, where the cultivated stands can produce at the rate of 2.5 to 5 tonnes per hectare. World starch production from cereals is about 1,360 million tonnes so the 2.2 million hectares of sago palm could produce only some 1.5 per cent of world production, but in some areas the contribution from sago palm could be important. In Sarawak, for instance, the palm is an important crop in peaty soils, and in swampy areas in the tropics sago palm will grow quickly (8 years to maturity compared with 13 years in other areas). In food energy terms the palm will out-yield any other crop.

Professor Flach does not see pure sago starch as a challenge to the central role of rice, but in Indonesia more and more people are eating bread, and sago starch added to bread flour can significantly improve quality. The country imports around a million tonnes of grain a year, and any application of the indigenous sago palm that would help reduce imports would be of vital importance.

Most of the work on sago palm is directed towards production in Indonesia, where the ministry of public works is studying the potential use of coastal swamps in sago growing. And now there is a major project sponsored by the World Bank. "Suddenly nearly everybody is working in the field", says a surprised Professor Flach.

Casper Schuurin

Software copyright

MITI retreat on "piracy"

Tokyo

JAPAN'S Ministry of International Trade and Industry (MITI) has abandoned its controversial plans to make changes in the laws governing software copyright — changes which, in the US view, would have made "worldwide software piracy legal". But plans are going ahead rapidly to strengthen the software industry by setting up a new ¥ 290 million (£900,000) database research centre and by merging software organizations into a single promotional group.

What worries MITI is that Japan's software industry is not growing sufficiently rapidly to enable it to catch up with the West. In the United States and Europe, the explosive growth of the software industry has been fired by the appearance of huge numbers of small entrepreneurial software houses selling software packages wherever a niche can be found for them. Japan is only just entering this stage. Until now, growth has been hampered by big businesses which want to construct their own software or have its specially built for themselves alone, rather than buying a package off the shelf.

To try to boost the industry, MITI called for a new law "more clearly to protect the rights of computer programmers" and to give programmers the right to "allow or refuse the use of software by a third party". At the moment, software rights are provided in an ill-defined way by copyright laws administered by the Ministry of Education, Culture and Science (MESC)'s Cultural Affairs Agency.

But instead of the fifty years' protection given under Japanese copyright law or the seventy-five years given under US law, MITI proposed that only fifteen years be guaranteed — even though some of the early US software still in use is already about that old. What is more, the new law made it possible to require a software developer to sell his software in exchange for a "reasonable payment", whether or not he wanted to sell it, when the sale was "in the public interest" or when use was refused without a "justifiable reason".

Despite strenuous denials from MITI that the plan had been thought up to help Japanese companies to get access to US software technology, the US software industry reacted with fury. Many also felt that the successful lawsuit brought by IBM against Fujitsu and Hitachi — two of Japan's top computer makers — which forced them to pay huge sums in compensation for allegedly copying and reselling IBM software had stirred MITI into proposing such a radical change in the law.

Joining in the US protest was MESC's Cultural Affairs Agency, which refused under any circumstances to yield its control of software copyright to MITI. The combined opposition was eventually sufficient

to overwhelm MITI, but not before a remarkable series of semantic squabbles between MESC and MITI over whether software was akin to literature, and thus clearly material that should be protected by copyright, or an "industrial process" which MITI could claim should be dealt with by its patent department.

While MITI's plans for new software laws have reached a dead end, progress has been made this past week in regrouping Japan's database research and development groups. MITI is to use funds from one of its more profitable sidelines — running the nation's bicycle races — to draw together the fragmented database industry. Under the bicycle race law, MITI supervises the "fair and safe conduct of all bicycle races" and puts the profits into the improvement of "bicycle and mechanical technology" as well as improvements in "medical care and public hygiene". Although funding databases may be stretching the law's original intentions, ¥170 million has been earmarked from the fund as an initial payment to help set up a new institute for database research. A further ¥120 million is to be provided by a group of 20 industrial companies, including Mitsubishi, Hitachi, Toshiba, NEC and Fujitsu, that MITI is bringing together along with a number of academic researchers in the universities to form the new group.

The aim is to bring database development up to Western levels. MITI was

spurred into action by the result of a survey showing that 679 databases were in use in Japan — ranging from economic statistics to chemical literature — but only 157 (23 per cent) of them had been developed inside the country. Major problems have yet to be overcome, however, to bring together the different groups working on databases. Some databases are already being developed in universities and in the Japan Information Centre of Science and Technology with support from the Science and Technology Agency.

A large database is also under construction in MITI's patent office to store patent information. As it covers a very wide range of fields, it is essential that it be properly integrated into the overall strategy. The problem is the familiar one in Japan of getting competing ministries to work together — particularly difficult in this case because part of MITI's aim in setting up the group was to establish that databases belonged in its territory rather than that of the Ministry of Posts and Telecommunications. The problems are expected to be resolved by the summer and the location and structure of the centre to be agreed upon.

A new industrial grouping is also almost ready for those involved in other areas of software development. Both personal and commercial software are now to be grouped together into one umbrella organization under MITI's aegis to provide a centralized group capable of more "organic" activity. The details of the new organization will be released by MITI at the end of the month.

Alun Anderson

Hungarian science

Making the most of society

HUNGARIAN biology must come to terms with the challenge of computers, information technology and systems analysis, Dr Janos Szentagothai, president of the Hungarian Academy of Sciences, told the academy's general assembly last month. The theme of the meeting was the relationship between biology and society — an important subject in a country with a considerable agricultural and food industry and environmental legislation going back to the mid-nineteenth century.

Dr Szentagothai concentrated on three main topics: the academy's role as scientific coordinator in the preparation of socio-economic decisions, basic research of "outstanding future importance" and participation in certain aspects of scientific public life. He stressed the setbacks suffered by Hungarian science as a result of the swingeing cuts in research expenditure two years ago. Even a year's moratorium on spending on new equipment and the servicing of equipment already purchased from abroad had, he said, greatly hindered the utilization of the country's leading intellectual resources.

During the meeting, Deputy Premier Jozsef Marjai announced a special government credit of 200 million forints (£33 million) for research, in addition to what had been originally planned, most of it to be allocated by the academy for basic research. Nevertheless, Dr Lenart Pal, the general secretary of the academy, urged that other ways of generating research funds must be encouraged, such as the formation of "profitable partnerships and subsidiaries" by research institutes and industry.

It was, however, what Dr Szentagothai called "scientific public life" which provided the major innovation this year. Starting in 1985, the academy's elections are to be "democratized", with multiple candidates for each elective post (president, vice-president, presidium members) and tenure normally restricted to two consecutive five-year terms. A 14-person nominating commission, headed by Professor Bruno Straub, was elected by this year's assembly, and will begin work next March on preparing the necessary lists of candidates. **Vera Rich**

Creation research and Noah

SIR — Jukes's commentary on his caricature of creation (*Nature* 308, 398–400; 1984) is a superb illustration of the unscientific approach of the anti-creationists, especially his conjuring up of "data" to support his polemic. Contrary to Jukes's demonstrably false claims, I have never sent graduate students to do field research on fossil human footprints (although I have led them on Grand Canyon field studies), have never even considered specializing in the "ecology of Noah's Ark", and certainly did not (indeed, could not) synthesize the Taylor's sophisticated museum display with which I am pictured. In the "half (or one-tenth) truth" category, Jukes labours the fact that I wrote a children's book on fossils, but mentions neither that the book was written for Christian families (not at all for public schools) nor that I have co-authored two two-model books with quite a different approach for public schools and, with a highly respected biologist, four widely used college programmed textbooks in biology. His misrepresentation of my colleagues and other aspects of creation science are even further off the mark.

If Jukes (unfortunately rather typical of the anti-creationists) cannot be trusted accurately to report simple, easily obtainable facts verifiable in the present, how can anyone trust his wild-eyed speculations about the complex and unobservable past? It is sad to see a man of Jukes's former stature sink so low. But if *Nature* (a refereed journal?) and those evolutionists who are scientists want to retain a claim to fair and objective scientific rationality, they must separate themselves publicly from Jukes's pitiful tantrum. At least Jukes's article will make thinking people everywhere wonder what is wrong with eliminating "the current methods for teaching evolution" if he exemplifies them.

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● Jukes replies — Professor Parker incorrectly states that I wrote a caricature of "creation". My article was about creationism, a rather different topic.

Parker says he has "never sent graduate students to do field research on human footprints". However, this topic of graduate study is listed in the Institute for Creation Research (ICR) General Catalog (p.14). His "Grand Canyon field studies" would presumably be related to his assertion (Parker, *Dry Bones*, pp.53–54) that the canyon was formed 5,000 years ago by the Great Flood. Although he "did not . . . synthesize the Taylor's sophisticated *sic* museum display", an essentially similar pictorial representation appears in *Dry Bones* p. 24.

Parker's speciality on the ecology of Noah's Ark was inferred by me from *Dry Bones*, which devotes about 16 pages to Noah's Ark and the flood, with pictorial details of the fauna and their food supply.

Parker says I did not mention that *Dry Bones* was written for Christian families. The book is offered for public sale in the ICR book catalogue, where it is described as "fun as well as educational". Despite Parker's assertions, *Dry Bones* has been used for science instruction in at least one public school, where it aroused protest by families that include Christians. I did not find it necessary to publicize his other literary efforts.

I made no misrepresentation of his colleagues; I let them speak for themselves. Parker speaks of my "wild-eyed speculations about the complex and unobservable past". The best response is to quote some of Parker's own pronouncements. For example:

"If the fossils are the plants and animals drowned in Noah's Flood, then they would all be about five to seven thousand years old." (*loc. cit.*, p. 44).

"Before the Flood there were many more plants without seeds compared to seed plants. Plants without seeds didn't survive the Flood as well seed plants."

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Semen and AIDS

SIR — In the letter "Semen and AIDS", by G.M. Shearer and A.S. Rabson (*Nature* 15 March, p.230), some consequences of the physiology of mammalian reproduction appear to have been overlooked.

It is true that semen is deposited in the vagina which is lined by stratified, squamous epithelium, but to reach the ovum sperms have to ascend through the endocervix which is lined by tall columnar (picket) epithelium. This is less likely to be traumatized than the rectal mucosa of a catamite, but its penetration by sperms has been invoked in explaining the origin of carcinoma, while anti-sperm antibodies are common in the human female.

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Fetal viability

SIR — Dr Jansen's choice of definition of "previable" is not as plain as he thinks (*Nature* 26 April, p.768). The *de facto* legal definition of viability is currently 28 weeks in the United Kingdom. The National Health and Medical Research Council (NH & MRC) of Australia suggests 20 weeks; others suggest 22 or 24 weeks as the medical definition of viability. If there is "a point at which extrauterine existence is possible", it

would appear to extend over an eight-week period, which is a mathematical contradiction. But, of course, there is no "point", not even 20 weeks, because NH & MRC had to provide a rider to this choice when it stated that "dissection of the fetus should not be carried out while a heartbeat is still apparent or there are other obvious signs of life".

Dr Goodhart's choice of definition (*Nature* 26 April, p.768) appears more reasonable but on analysis is seen to contain a major flaw. The span of human existence from conception until death may be divided into 9 months in a fluid environment and the remainder in an air environment. It is axiomatic to state that the fetus flourishes in its fluid environment, even if it may die in that environment. It is illogical, therefore, to relate the viability of the fetus to its unnatural extrauterine state, just as it would be illogical to relate the viability of those born to their ability to survive in a fluid environment once again.

The best definition of the term "viable" in relation to the embryo or fetus that I could find is "capable of maintaining life". The term "previable" adds nothing to our understanding of intrauterine human existence. PATRICK W. GILL
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Biological control

SIR — May I add to the list of methods suggested for control of the fly in a domestic environment? Curiously, neither F.E.G. Cox¹ nor K.L. Bell² have considered the possibility of biological control. There are several advantages to be gained from establishing a flourishing domestic population of the fly's natural predator, the spider. In particular, application of this means of control would overcome the moral dilemma posed by the choice between swatting the fly and forcibly expelling the insect into an often hostile world.

As in all habitats, it is important to maintain a correct ecological balance within the home or laboratory. Nevertheless, my proposal is preferable to a more drastic form of biological control recorded many years ago. As pointed out by R. Bonne³, direct predation of flies by humans (particularly elderly females) would seem to be unwise. It can be the beginning of a surprising food chain which, if this anecdotal account is to be believed, can have serious medical consequences and can even result in death in extreme cases.

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1. Cox, F.E.G. *Nature* 307, 8 (1984).

2. Bell, K.L. *Nature* 308, 684 (1984).

3. Bonne, R. *There was an Old Lady who Swallowed a Fly* (Southern Music Publishing Co. Ltd.).

Blood group and socio-economic class

A report last year, based on a study of a British population, that A blood groups are significantly more common among members of the higher socio-economic groups (classes I and II) has generated a wealth of correspondence, some of which appears in what follows together with a reply from the original authors. The general conclusion seems to be that there can be no general conclusion.

BEARDMORE and Karimi-Booshehri¹ recently reported an association between ABO blood group phenotype and social class. They found that in both sexes the A phenotype was significantly more frequent and the O phenotype significantly less frequent in social classes I and II, the converse being true for social classes III, IV and V. In an attempt to replicate Beardmore and Karimi-Booshehri's findings we have analysed data obtained by the National Child Development Study (NCDS)².

The NCDS study commenced as the Perinatal Mortality Survey and involved all children born in Britain during the week 3–9 March 1958. At that time detailed obstetric data were obtained on each child^{3,4}. In addition the mothers blood type (ABO and Rh status), her occupational status at commencement of pregnancy, and the occupational status of her father and of her husband were noted. The standard geographical region in which she lived and in which she was born were also recorded.

The occupational status of a married woman can be classified in several ways: (1) by her own socio-economic group (although this necessarily excludes a large number of women who are housewives); (2) on the basis of her father's occupation (when she left school); (3) on the basis of her husband's occupation (in 1958). We have used all three classifications in analysing the relationship between ABO blood group phenotype and social class.

Table 1 presents the breakdown of ABO blood group phenotype by social class and by migrational status. Natives were defined as those born and still living in the same region; all other individuals were classified as migrants. The regions were defined as the 11 standard regions of Britain⁵.

For all three classifications of social class we found no evidence to support the notion of either heterogeneity between migrants and natives ABO phenotypic distributions (see Table 1) or an excess of the A phenotype in social classes I and II.

There are several possible reasons for our failure to replicate Beardmore and Karimi-Booshehri's results:

Table 1 Percentages of ABO blood group phenotypes by social class (three classifications) in natives and migrants in Britain

		Social class					Total		
Classification		Blood group	I	II	III	IV	V	No.	%
Mother's own	Migrant	A	41.7		39.4	44.4	50.0	327	41.6
		O	50.0		49.8	43.7	42.9	379	48.2
		B	7.6		6.9	9.3	5.4	58	7.4
		AB	0.7		3.9	2.6	1.8	23	2.9
		Total no.	144		436	151	56	787	
	Native	A	40.8		42.3	41.5	47.6	1,329	42.2
		O	47.8		46.6	48.6	44.7	1,479	47.1
		B	8.0		8.5	8.2	5.8	257	8.2
		AB	3.3		2.7	1.7	1.9	77	2.5
		Total no.	299		1,832	805	206	3,142	
χ^2 testing: ABO by social class (migrant)			$\chi^2 (9) = 8.359 P = 0.498$						
ABO by social class (native)			$\chi^2 (9) = 7.226 P = 0.614$						
ABO by migrational status			$\chi^2 (3) = 1.306 P = 0.728$						
Migrational status by social class			$\chi^2 (3) = 54.963 P < 0.001$						
Father's social class	Migrant	A	36.2	38.8	41.5	41.7	45.7	718	41.1
		O	59.4	53.0	46.0	47.4	42.6	835	47.9
		B	0.0	4.7	8.7	7.8	7.8	129	7.4
		AB	4.3	3.5	3.7	3.2	3.9	63	3.6
		Total no.	69	317	882	348	129	1,745	
	Native	A	40.4	40.9	41.2	43.6	41.7	2,538	41.8
		O	47.5	47.4	47.5	45.4	45.0	2,836	46.7
		B	10.1	8.5	8.4	8.2	10.3	521	8.6
		AB	2.0	3.2	2.9	2.8	3.0	176	2.9
		Total no.	99	709	3,054	1,585	624	6,071	
χ^2 testing: ABO by social class (migrant)			$\chi^2 (12) = 17.320 P = 0.138$						
ABO by social class (native)			$\chi^2 (12) = 6.551 P = 0.886$						
ABO by migrational status			$\chi^2 (12) = 5.062 P = 0.167$						
Migrational status by social class			$\chi^2 (4) = 110.064 P < 0.001$						
Husband's social class	Migrant	A	37.8	42.2	40.9	45.4	41.2	805	41.4
		O	51.9	46.8	46.4	45.4	43.8	910	46.9
		B	8.9	8.7	9.3	6.9	12.5	173	8.9
		AB	1.4	2.3	3.4	2.3	2.5	54	2.8
		Total no.	214	393	993	262	80	1,942	
	Native	A	43.4	43.4	41.4	40.8	39.2	2,855	41.5
		O	48.4	45.4	46.8	48.6	48.6	3,243	47.1
		B	5.5	7.2	8.9	8.1	8.7	576	8.4
		AB	2.7	4.0	3.0	2.5	3.5	209	3.0
		Total no.	256	917	3,991	1,293	426	6,883	
χ^2 testing: ABO by social class (migrant)			$\chi^2 (12) = 9.350 P = 0.673$						
ABO by social class (native)			$\chi^2 (12) = 13.629 P = 0.325$						
ABO by migrational status			$\chi^2 (3) = 0.874 P = 0.832$						
Migrational status by social class			$\chi^2 (4) = 248.342 P < 0.001$						

Values in parentheses are d.f.

(1) We have looked only at women, whereas Beardmore and Karimi-Booshehri considered both sexes. Nevertheless it is clear from their analysis that their effect is found in both sexes to the same degree. We have attempted to repeat their study by considering both natives and migrants. If this restriction is removed, the sample available for analysis increases, but there still remains no association between ABO blood group status and social class.

(2) The NCDS is a sample of the breeding population of Britain whereas the blood donor data will also include non-breeders. However, it is more likely that the sampling procedure used by the NCDS generates a truly random sample, whereas it is not inconceivable that blood donor data could be biased.

(3) Some evidence of nonrandomness in the blood donor data can be obtained from analysis of the social class distributions. Comparison of each data set with the Registrar General's social class distributions indicates that the NCDS data do not differ markedly from expectation whereas the donor data reveal a significant excess of people in social classes II and V (about 5% and 12% respectively) and relative deficiency of 21% in social class III. There is also marked heterogeneity between NCDS and blood donor samples, for example, using the husband's occupation $\chi^2 = 1,746.855$, $P < 0.0001$ (d.f. = 6). Restricting the comparisons to the Registrar-General's standard regions of East and West Riding and south-west England does not affect the above conclusions.

We also note that Beardmore and Karimi-Booshehri found an association only between ABO phenotype and social class and not between migrational status and ABO phenotype, despite a clear association between migrational status and social class.

(4) The ABO phenotype frequencies differ between samples. This is to be expected since we have analysed data from Britain as a whole rather than two geographical regions. However, if we restrict our analysis to the two Registrar-General standard regions mentioned above there is no heterogeneity in ABO phenotypes between samples. Even with this reduced sample we can find no evidence to support Beardmore and Karimi-Booshehri's results.

We have also examined the relationship between social mobility and ABO phenotype, classifying mothers on the basis of her own and her father's social class, as either upwardly mobile, downwardly mobile or non-mobile. Again there was no evidence that this intergenerational social mobility was related to ABO phenotype ($\chi^2 = 1.457$, $P = 0.962$, d.f. = 6).

We thank Mr K. Fogelman, and the National Children's Bureau, for allowing us to re-analyse the data obtained by the National Child Development Study, and

Mr E. Roughley, and the Social Science Research Council's Survey Archive for supplying the data.

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1. Beardmore, J. A. & Karimi-Booshehri, F. *Nature* **303**, 522-524 (1983).
2. Davis, R., Butler, N. R. & Goldstein, H. *From Birth to Seven* (Longman, London, 1972).
3. Butler, N. R. & Bonham, D. G. *Perinatal Mortality* (Livingstone, Edinburgh, 1963).
4. Butler, N. R. & Alberman, E. D. *Perinatal Problems* (Livingstone, Edinburgh, 1969).
5. *General Register Offices Census 1961 General Report* (HMSO, London, 1968).

In a recent article¹, Beardmore and Karimi-Booshehri showed that among 10,000 blood donors from south-west England and Yorkshire there were marked differences in the ABO blood

group distribution with social class. The study by Mascie-Taylor and McManus shows an analysis of women who gave birth in the 1 week's national birth survey in 1958. Here we analyse data from a subsequent birth survey. The data relate to 14,384 women resident in Great Britain and who gave birth in the week 5-11 April 1970. The data were collected in the British Births survey^{2,3} sponsored by the National Birthday Trust Fund. Well over 95% of all births in England, Scotland, Wales and Northern Ireland were included.

The ABO blood groups of these mothers were compared with their social class, based on the occupation of the husbands, using the Registrar General's Classification of Occupations⁴. Unmarried mothers and those whose husbands' occupations were unclear were omitted ($n = 415$). The distribution of ABO blood groups using this classification is shown in Table 2. As shown for the 1958 survey, there was no significant difference between the social classes.

This classification uses the husband's occupation. We did not use that of the woman herself since married pregnant women are often housewives or are only in part-time employment. Information was available, however, concerning the

Table 2 Distribution of ABO blood groups of mothers delivering during 5-11 April 1970 by social class (based on husband's occupation)

Blood group of mother	Social class					All known
	I	II	III	IV	V	
O	368 (48.0%)	822 (47.0%)	4,110 (47.6%)	1,099 (49.2%)	484 (49.0%)	6,883 (47.8%)
A	300 (39.1%)	727 (41.4%)	3,482 (40.3%)	833 (37.3%)	373 (37.7%)	5,715 (39.7%)
B	73 (9.5%)	160 (9.1%)	792 (9.2%)	231 (10.3%)	108 (10.9%)	1,364 (9.5%)
AB	26 (3.4%)	45 (2.5%)	258 (2.9%)	70 (3.2%)	23 (2.4%)	422 (3.0%)
All known	767 (100.0%)	1,754 (100.0%)	8,642 (100.0%)	2,233 (100.0%)	988 (100.0%)	14,384 (100.0%)

$\chi^2 = 16.1$, d.f. = 12, not significant.

Table 3 ABO blood groups according to highest educational qualification of mothers

Highest educational qualification	Blood group				All known
	O	A	B	AB	
None	2,884 (46.9%)	2,510 (40.8%)	574 (9.4%)	180 (2.9%)	6,148 (100.0%)
Vocational	778 (49.0%)	623 (39.2%)	142 (9.0%)	45 (2.8%)	1,588 (100.0%)
O-level or equivalent	932 (47.3%)	816 (41.5%)	162 (8.2%)	59 (3.0%)	1,969 (100.0%)
A-level or equivalent	186 (47.2%)	154 (39.1%)	47 (11.9%)	7 (1.8%)	394 (100.0%)
SRN or Certificate of Education	272 (49.6%)	211 (38.4%)	52 (9.4%)	14 (2.6%)	549 (100.0%)
University or college degree or other higher	181 (44.8%)	173 (42.8%)	40 (9.9%)	10 (2.5%)	404 (100.0%)
All known	5,233 (47.3%)	4,487 (40.6%)	1,017 (9.2%)	315 (2.9%)	11,052 (100.0%)

$\chi^2 = 12.7$, d.f. = 15, not significant.

educational qualifications of the mothers of the surviving children who were followed up at the age of 5 yr.

As shown in Table 3, there is no evidence for a trend. The evidence of Beardmore and Karimi-Booshehri¹ would suggest that, as the educational qualifications increased, so the proportion of blood group O would decrease, and that of A would increase. True, in our data there were slightly more women with university degrees who had blood group A but this was far from statistically significant ($\chi^2 = 0.5$, d.f. = 1).

What, then, might account for the differences between the two studies? Both, of course, have selection biases—in Beardmore and Karimi-Booshehri's data, blood donors only are included and housewives are excluded. In our study, women who are infertile will be excluded and those with high fertility will be more likely to be represented. Although we have no information on the ABO distribution of the former, it has been shown⁵ that there is little variation in the distribution of ABO blood groups with the number of pregnancies the woman has already had.

From the data presented here we suggest that, although perhaps true of non-migrant blood donors in Yorkshire and the south-west of England, the finding of more blood group A amongst the upper social classes cannot be extrapolated to the female population as a whole. We know that data from the 1958 birth survey (see above) substantiates our findings. We therefore suggest that the original results¹ are likely to have been due to one of those random statistical variations which must occur by chance.

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1. Beardmore, J. A. & Karimi-Booshehri, F. *Nature* **303**, 522–524 (1983).
2. Chamberlain, R., Chamberlain, G., Howlett, B. & Masters, K. *British Births 1970* Vol. 1 (Heinemann Medical, London, 1975).
3. Chamberlain, G., Philipp, E., Howlett, B. & Claireaux, A. *British Births 1970* Vol. 2 (Heinemann Medical, London, 1978).
4. Registrar General of England & Wales *Classification of Occupations* (HMSO, London, 1970).
5. Butler, N. R. & Bonham, D. G. *Perinatal Mortality* (eds Livingston, E. & Livingston, S.) (Livingstone, Edinburgh, 1963).

FROM a χ^2 analysis done on an historic (1953–63) data base, Beardmore and Karimi-Booshehri¹ conclude that attributes influencing occupational type, social mobility and social class result from pleiotropic effects of the ABO alleles (or genes closely linked with them). In the most cogent of their theoretical citations, Herrnstein² suggests a possible heritable relationship between social class and intel-

ligence. The authors also cite Arthur Jensen's work³. Many recall the social upheaval which accompanied those writings a decade ago and seek to be very parsimonious when making ties between genetics and social class. In that context, I suggest that while the author's hypothesis may be true, it is not yet clearly indicated. Their extensive data seem better interpreted with the notion that there is no direct path from these genes to the behavioural phenotypes. In earlier work concerned with single gene substitutions and behaviour, I have supported the position that while single genes may sometimes have major effects, they often affect many forms of behaviour and that particular psychological traits are frequently influenced by many genes⁴. In the service of that theoretical viewpoint, I have reassembled and recalculated the data on ABO phenotypes as they assort by socio-economic group.

The highly significant outcomes reported¹ do represent real findings with regard to differential distribution of the ABO blood phenotypes across the five socio-economic job categories chosen. However, the authors have repeatedly combined categories to give classes I+II and III+IV+V. They also present the results by comparing the frequency of A phenotypes to the O+B+AB phenotype combination across the socio-economic categories. According to conventional usage in the behavioural sciences⁵ information is lost by these procedures. Groups may be (sensibly) combined for an analysis, of course, but this is normally done in order to meet the restrictions regarding minimum expected frequency requirements⁵. With these data, even the least frequent phenotype (AB) provides sample sizes that are adequate for independent χ^2 tests involving all five job categories. Then, since the χ^2 distribution is insensitive to the effects of order, a presentation of the contingencies by cells shows up areas of particular effect. Table 4 gives the obtained frequency divided by the expected frequency ratios for the four ABO phenotypes. It will be seen that for each phenotype there are one or two contingencies which indicate either marked under- or over-representation. The A phenotype is heavily over-represented in job category I and it is marginally high in category II. Phenotypes O, B and AB are all much under-represented in category I but show very different distribution patterns across categories II to V. The AB phenotype is low for category II as well as I and is notably high for category V. The test for the contingencies of Table 4 is significant: χ^2 (d.f. = 12) = 83.03, $P < 0.001$.

This significant statistical outcome, and those of the original report, may represent what can be called 'behavioural pleiotropy'. They do not confirm true pleiotropy, as that term has had meaning in formal genetics. In his classic text Grüneberg⁶ discussed genuine and what

Table 4 Obtained frequency/expected frequency ratios in the total data set of Beardmore and Karimi-Booshehri

Blood phenotype	Socio-economic category				
	I	II	III	IV	V
A	1.34	1.09	0.96	0.99	0.92
O	0.74	0.91	1.06	0.98	1.06
B	0.74	1.08	0.92	1.10	1.02
AB	0.78	0.84	1.00	0.99	1.18

he called 'spurious' pleiotropy. The rule is that in genuine pleiotropy a single gene controls two or more different traits which it produces directly by the use of different mechanisms. In spurious pleiotropy the gene does two or more things through the same mechanism or else has one effect which causes other things to happen. For example, in mice, A^y , a single gene which has genuine pleiotropic structural effects, for example, increased fat deposition, skeletal enlargement, linear size increase, also shows behavioural (spurious) pleiotropy in that it is also associated with the amount of exploratory activity exhibited by C57BL/6J mice⁷. The genuinely pleiotropic traits sum to effect greater body weight which, in turn, accounts for 48% of the variance in activity when A^y/a mice are compared with aa controls⁷. Thus, the behavioural pleiotropy can be clearly shown statistically but has no genetic basis of its own.

So finally, if we evaluate all of the ABO phenotypes concurrently, both the statistical and the definitional points work together in our understanding (and lack of understanding) concerning how the class differences reported¹ come to be associated with the ABO blood antigen system. We see that one blood phenotype is at comparative advantage for employment, another (the AB phenotype) is at comparative disadvantage, and that (especially in categories II–V) there is a great deal of intra-phenotypic variability across the socio-economic groups. This last point tempers the positive finding that the greatest part of the overall effect is seen at the extremes of the socio-economic continuum.

Thus, it seems a long way from the statistical outcomes to the privilege (or poverty) of class-based experiences, activities, comfort and substance as these subjects have matured from birth to their time of blood donation. In short, when we can show that some genotypically influenced mechanism intervenes between the socio-economic continuum and the discrete phenotypes of the ABO blood system, then we can show pleiotropy. When no mechanism is evident and a statistical outcome is our only basis, then social, cultural or other environmental influences seem more likely to contribute much of the variance to the behavioural pleiotropy found.

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1. Beardmore, J. A. & Karimi-Booshehri, F. *Nature* **303**, 522-524 (1983).
2. Herrnstein, R. J. *IQ in the Meritocracy* (Atlantic-Little, Brown, Boston, 1973).
3. Jensen, A. R. *Educability and Group Differences* (Harper & Row, New York, 1973).
4. Hawkins, J. D. in *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype* (eds Lindzey, G. & Thiessen, D. D.) 139-159 (Appleton-Century-Crofts, New York, 1970).
5. Siegel, S. *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956).
6. Gruneberg, H. *The Genetics of the Mouse* (Cambridge University Press, 1943).
7. Hawkins, J. D. in *Behavioral Genetics: Method and Research* (eds Manosevitz, M., Lindzey, G. & Thiessen, D. D.) 502-506 (Appleton-Century-Crofts, New York, 1969).

THE conclusion of a recent paper¹, that a pleiotropic effect of ABO alleles or linked genes can influence occupational type, seems to be premature. The authors assume that occupational classes are homogenous from an ethno-historical point of view, assortative mating among social classes is not significant, the ABO distribution of donors is similar to that found in the general population, selection on the ABO system is not differently operating in social classes and finally, that the occupation as a variable is the most representative one that defines the stratification of a society.

In Chile, as the authors mention, there is a clear socio-ethno-genetic cline which originated at the Spanish Conquest in 1541^{2,5}. The highest socioeconomic strata of urban areas have gene frequencies for ABO, Rh and other genetic markers similar to those found in Europe, the lowest ones have gene frequencies similar to those expected for a half European-Chilean Aboriginal admixture; middle classes have intermediate values⁶. This cline is present independently of the last two or three generations of European immigrations^{7,8}. Thus the problem is to explain why such a cline has persisted for 17 generations. Aboriginal people disappeared a few generations after the Spanish Conquest. Even 20% socioeconomic random mating should have cut down these gene differences but a strong socioeconomic assortative mating was found in the population of Santiago⁹. This seems to be the explanation for the stability of the socio-genetic cline. There is no reason to assume that a similar mechanism is not operating in England. Since geographical population heterogeneity (like that found also in Chile) due to invasion, settlement and migration is accepted by the authors, it would be necessary to demonstrate that ethnic heterogeneity and assortative mating among occupational classes cannot explain the observed ABO heterogeneity, before accepting the authors' hypothesis.

Blood donors may not represent either

the social or the genetic structure of their populations of origin. This could not only be due to higher requirements for O and Rh⁻ blood groups as the authors assume. If the relationship between ABO and diseases is accepted, and donors are relatives or friends of patients that need transfusions, the ABO distribution of donors may differ from that of the whole population. The authors do not describe the system to call for blood donors. Relatives of patients would increase distortions due to the ABO-disease relationships, colleagues or co-workers of patients would yield a distribution similar to that of the patient social class. These two types of calling donors or other ones, may be differently distributed in occupational classes, thus leading to different ABO distributions among them. We could not use the donors ABO distribution in the high socioeconomic classes because it was different from the mother-infant distribution in a study on socio-economic assortative mating⁹. Moreover, ABO-associated diseases might interact with social classes in an unpredictable way. Furthermore, natural selection operating on the ABO system¹⁰ may differ among social classes, not necessarily at present, but along the history of these classes.

Table 1 of ref. 1 shows that in class I, A migrant donors are less frequent than A native donors (z for proportions⁸ = 2.14, $P < 0.017$ in south-west England; $z = 1.2$, $P < 0.11$ in Yorkshire; $z = 2.25$, $P < 0.013$ in both places together); thus non-A migrant donors are fitter than non-A native donors to be found in class I. This suggests rather a socio-genetic interaction and not a simple gene action on work abilities.

Even accepting that a genetic action associated with ABO system influences the probability of reaching a determined social class, it is not possible to conclude that this gene action is related to skilled/creative or skilled/repetitive occupations. Several other social variables may influence the occupational class an individuals belongs to, such as culture, power, prestige, income and life-style, the latter being rather properties of class and conditioning individual occupation or social mobility. If so, the ABO-occupational class relationship may be true, but only as a circumstantial association.

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1. Beardmore, J. A. & Karimi-Booshehri, F. *Nature* **303**, 522-524 (1983).
2. Pinto-Cisternas, J. *et al. Hum. Hered.* **21**, 431-439 (1971).
3. Rothhammer, F. *Rev. Med. Chile* **101**, 247-251 (1973).
4. Palomino, H. *Rev. Med. Chile* **104**, 371-374 (1976).
5. Rothhammer, F. & Cruz-Coke, R. *Curso Básico de Genética Humana* (Editorial Universitaria, Santiago, Chile, 1977).

6. Workman, P. L. in *Methods and Theories of Anthropological Genetics* (eds Crawford, M. H. & Workman, P. L.) 117-150 (University of New Mexico Press, Albuquerque, 1973).
7. Costa, R. thesis, Univ. Chile (1966).
8. Valenzuela, C. Y. & Harb, Z. *Am. J. hum. Genet.* **34**, 925-936 (1982).
9. Valenzuela, C. Y. & Harb, Z. *Soc. Biol.* **24**, 225-233 (1977).
10. Mourant, A. E. *Ann. hum. Biol.* **9**, 575-577 (1982).

BEARDMORE and Karimi-Booshehri¹ conclude that "The only apparently reasonable explanation" for the distribution of the I^A allele is that it "confers on those who possess it... abilities significant in determining socio-economic class" (pleiotropy and linkage being gratuitous qualifiers). No less disingenuously, they assert that "social mobility in the United Kingdom has been, for some generations, a factor which would tend to homogenize initially different gene pools". The question is for how many more generations did social immobility increase the heterogeneity of gene pools? An order of magnitude more than "some"? And how quickly would Karimi-Booshehri and Beardmore expect that heterogeneity to disappear? Figuratively overnight? Apparently.

People who inherit wealth do not start at the starting-line with the rest of the runners, nor do their children or their children's children. It is reasonable to speculate that the present distribution of the I^A allele is one time-frame taken from a gradual sequence of allelic desegregation—the remnant of centuries of tradition during which the mere possession of wealth and social class was enough to ensure the maintenance of same—even in the face of a conspicuous lack of ability, genetic or otherwise.

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BEARDMORE AND KARIMI-BOOSHEHRI REPLY—Mascie-Taylor and McManus's interesting findings on a sample of mothers fail to confirm ours. They consider a number of possible reasons for this. However, they do not take age into account. As the majority of children are born to mothers in their twenties or early thirties there is massive overrepresentation of younger cohorts in their sample. Thus, quite apart from the omission of non-breeders, their sample could not be said to be representative of the female segment of the population in a social sense.

A second important difference in sample compositions between the two

studies is that in Mascie-Taylor and McManus' data for geographical regions are pooled. As there are both average genetic and average socio-economic differences between regions the pooling makes comparisons very difficult.

It is true that our data are nonrandom in terms of social class proportions. We are unable to judge the effect of our exclusion of cases with no or ambiguous occupational labels but it would not be surprising if this produced under-representation of class III especially as one of the commoner labels excluded was 'civil servant'. However there is no obvious mechanism by which such nonrandomness should lead to the results observed.

Mascie-Taylor and McManus are concerned that we have not demonstrated an association between migrational status and ABO type and believe that this may imply mis-classification of social class. Our view is that we have, in this study, been at very considerable pains indeed to avoid mis-classification and that mis-classification is likely to have been at a very low level and lower than other relevant studies. Furthermore it does not follow that an association between migration and ABO type would be detectable as the influences on migration and social class are by no means coincident.

There is a major difficulty in comparing the data of Golding *et al.* with ours. This is that they use the social class of husbands to assign social class to the women in their surveys. There is a correlation between social class of spouses but it is by no means perfect. We were meticulous in our assignment of social class from occupational type. It is interesting to note that recalculation of the total 1958 data ($n = 13,969$) collected in the First Perinatal Mortality Survey (of which the data given in Table 1 are a subset) shows the ratio $A/O + B + AB$ to be almost identical in form with our Fig. 1 (Spearman rank correlation in both cases is 0.9, $P = 0.05$). The pattern for the 1970 data quoted by Golding *et al.* is different and indeed the χ^2 testing homogeneity of the 1958 and 1970 data on ABO phenotype frequencies is very close to the 0.05 level of significance. The presence of Northern Ireland data in only the 1970 sample makes it difficult to assess whether it is this or temporal changes which may be responsible for the difference. The 1958 and 1970 samples also differ from each other in social class composition and this, too, complicates comparisons.

Hawkins raises two issues. One concerns the difference which he sees between 'true' and 'spurious' pleiotropy. This is a semantic point. While there may be a case for

Table 5 Proportions of ABO phenotypes in two samples from Aberdeen

Sample	Phenotype				N
	O	A	B	AB	
Donors	50.27	35.46	10.87	3.40	7,092
Schoolchildren	49.75	36.16	10.72	3.37	17,060

making distinctions as to types of pleiotropy, many geneticists, including the writers, would subscribe to the view that all effects (whether these affect anatomical, behavioural, biochemical, reproductive or viability aspects of the phenotype) of gene differences can legitimately be considered pleiotropic effects. The second point is concerned with the method of statistical analysis. Pooling of data always leads to loss of information but the main conclusions are unaffected in this example whether we pool or not.

We have partitioned the table into a series of 2×2 tables from each corner. Irrespective of the starting point, it is clear that O, B and AB do not differ over socio-economic class and therefore the table is collapsible in the sense of Bishop, Fienberg and Holland¹. Thus, only the differences $A/(O + B + AB)$ over social class are relevant. Using the partition method of Daly², comparisons which show significance (after pooling blood groups) are those between classes I and II and (I + II) against the rest. Clearly, the significance of the first requires further analysis, but the second accounts for about 50% of the overall χ^2 and cannot be controverted. We also noted a significant effect for classes (I + II + III + IV) against class V, but this only confirms the original conclusions.

Valenzuela takes the view that assortative mating within social classes derived from ethnically different populations is the proper explanation of our results. While ethnic heterogeneity is demonstrable in the Chilean population it is not apparent in the population from which our data are drawn. The difference in the proportions of the A phenotype in migrants and natives is, we think, significant and needs further investigation. In no way do we subscribe to the view, which Valenzuela appears to think we hold, that non-genetic factors are unimportant in determining occupation or other indices of social class.

The other question Valenzuela raises is that of how random a sample of a population is obtained by collection of blood donors. There is a clear need to test this and we hope shortly to examine this question using large samples of newborn and donors from the same region. Nonrandomness, if it occurs, will most likely take

the form of over-representation and under-representation of particular phenotypes and the influence of patients on recruitment to donor panels in the UK is unlikely to be significant. One relevant set of data is available in which the distribution of ABO phenotypes can be compared in donors and schoolchildren in one locality. This comes from the work of Allan³ and is given in Table 5. The phenotype distributions in the two samples agree very well and the very small difference between the two is in the expected direction of slightly more O in the donor sample. It is, of course, possible that such samples differ in social composition.

Hartung asks an important question which cannot be answered with assurance. We have, however, with the collaboration of Dr R. Gilbert, attempted to make some crude estimates which are necessarily based on a number of debatable assumptions. Let us suppose that mixing of two populations each polymorphic for genes B^1 and B^2 with the frequencies of B^1 being 0.35 and 0.15 respectively occurs. Even if the gene flow between the two populations ('classes') is as low as 2%, the two classes will have frequencies of B^1 of 0.26 and 0.24 respectively in 56 generations. With 5% gene flow the same frequencies are achieved in 22 generations. Using 25 yr as the length of a generation, the time lapse and gene frequencies are perhaps reasonably applicable to populations at the time of the Saxon invasions of the British Isles which had considerable consequences for power and class. The important point is that these levels of gene flow are very low and it is not unreasonable to suppose that effectively much higher levels of gene flow have operated both in the recent and more distant past, although there is no obvious way of testing this view.

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1. Bishop, Y. M. M., Fienberg, S. E. & Holland, P. W. *Discrete Multivariate Analysis* (MIT, 1975).

2. Daly, C. *Biometrics* 17, 114 (1962).

3. Allan, T. M. *Hum. Hered* 22, 578-583 (1972).

Psychoimmunology before its time

That there is probably a link between the central nervous system and the immune system is easily accepted. The doubt is whether enough is yet known to sustain people's hopes of explanation.

THE notion that emotional disturbance may trigger off physical illness is in no sense new. That people may be driven by adversity into decline is a familiar theme in literature. Strictly speaking, the phenomenon is distinct from that of psychosomatic illness such as Freud's well-documented cases of hysterical paralysis in which, in Freudian terms, the illness is a kind of talisman for the emotional disturbance. Instead, the physical consequences of emotional shock may become manifest in normal people even if they are more spectacular among those with an underlying emotional disturbance.

Over two decades, Professor G.W. Brown of Bedford College, University of London, has been collecting evidence that what he calls life-events (imprisonment, relocation or personal bankruptcy, for example) may provoke illness in outwardly normal people. The speed with which a person's death will follow that of his or her spouse is the most familiar occurrence of this kind. The pattern is familiar, but the mechanism is unknown.

That, at least, has been the state of affairs until quite recently. Now, as some of the new generation of medical students know perhaps too well, there is emerging a speciality called psychoimmunology — a means of explaining such phenomena as the increased risk of death on bereavement in terms of the disturbance caused to the immune system in the wake of emotional shock or deprivation. The difficulty, for the rest of us, is that of knowing just what weight to give to these accounts.

That there should be a functional connection between the central nervous system and the immune system is not in itself surprising. In animals, neurophysiologists now have a lot to say about the way in which the brain controls the system of locomotion, while the regulation of the reproductive system in mammals by means of the peptide hormones involved in the regulation of the pituitary gland by the hypothalamus is being understood in ever finer detail.

A kind of connection between the central nervous system and the immune system is made plausible by several unconnected observations, not the least of which is that some types of the lymphocytes involved in the body's immune response to, say, bacterial infections are found to carry receptors which recognize simple peptide hormones also found in the brain.

Some of the connections have been

reviewed by David Maclean and Seymour Reichlin (*Psychoneuroimmunology* 12, 475; 1981), and can plainly take several different forms. Maclean and Reichlin offer the interaction of the hypothalamus and the pituitary as the most interesting source of materials that may modify immune function. Some possible interactions are direct, as with the pituitary hormone that appears directly to stimulate the development of cells in the thymus, the organ in which T-cells become recognizably different from cells that produce antibodies. But the best-known routes by which pituitary hormones may affect the immune system are indirect, involving the stimulation of the adrenal cortex by hormones such as adrenocorticotrophic hormone.

This imbalance is unlikely to persist for long. As techniques improve for distinguishing between T-cells in different stages of their maturation and for sustaining them in culture, there has naturally been an upsurge of interest in the measurement of response by lymphocytes to hormones of various kinds. It seems to be established that the steroid hormones of the adrenal cortex more markedly stimulate lymphocytes at early rather than late stages in their differentiation, which may suggest how the composition of the circulating lymphocytes may be controlled by peptide hormones.

And then, it seems, some peptide hormones stimulate T-cells to produce lymphokines (such as interleukin-2 but also interferon) and others have the opposite effect. The more radical psychoimmunologists talk as if there is no state of mind which is not faithfully reflected by a state of the immune system.

Others go further. J. Edwin Blalock of the University of Texas at Galveston, writing in the *Journal of Immunology* in March (132, 1067; 1984) under the title "The immune system as a sensory organ", argues that the interaction between the central nervous system and the immune system must be reciprocal, with peptide hormones of the pituitary and lymphokines secreted by lymphocytes having similar functions in the blood and with antigenic stimuli such as different kinds of bacterial infections producing physiological and even behavioural changes in the infected animal. He does not quite say that the emotional consequences of measles and tuberculosis infections should be distinguishable — but of course, they are.

The other line of inquiry that has helped

put psychoimmunology on its feet are experiments with whole animals. In many ways, these are adjuncts of the classical investigations of the effects of stress on the system of adrenal hormones and thus on blood pressure, heart rate and so on.

In one typical investigation (Laudenslager, M.L. *et al. Science* 221, 568; 1983), groups of a dozen rats were given electric shocks of two kinds, and the sensitivity of their lymphocytes to artificially provoked cell division by the materials called mitogens assessed. The more seriously stressed rats were found to have the least easily stimulated lymphocytes at the end of the experiment.

Surprisingly, very similar investigations can be mounted with people. Thus S.J. Schleifer *et al.* have tackled directly the question of the immunological consequences of bereavement by means of a prospective study among men whose wives had been found to have breast cancer. Those who were eventually widowed have been reported (*J. Am. med. Ass.* 250, 374; 1984) to have had circulating lymphocytes less responsive to the effect of mitogens, just like the rats given electric shocks.

Investigations such as these, while time-consuming and costly, at least have the merit of being practicable and, once mounted, easy to interpret. The snag is that statistically significant samples are accumulated only slowly, and at a high cost.

Why, in these circumstances, does there persist a stout band of near-sceptics convinced that too much is being made, at this stage, of the explanatory value of psychoimmunology? There are several reasons, not the least of which is that very similar lines of argument, more accurately, hypotheses, have been used within living memory speciously to suggest links between a person's chance of developing cancer and his or her state of mind.

Second, because knowledge of T-cells and their function is growing so quickly, it is necessarily difficult to design systems for assaying the effects of hormones, or of states of mind, on lymphocytes that are reproducible and significant.

Third, one rather crucial question seems to have been left for too long aside. If it should be that a person's state of mind, say grief, may affect the quality of his immune system, how does it then transpire that he or she will be more likely to die of heart attack or stroke or even in a road accident? In short, the explanation is likely to be part of a more complicated tale. **John Maddox**

Evolution

Palaeontology at the high table

from John Maynard Smith

THE Tanner lectures, recently delivered by Stephen J. Gould of Harvard University, and followed by a panel discussion*, provide an opportunity to assess the current contribution of palaeontology to evolutionary theory. It might be supposed that this contribution would be crucial, but, at least until recently, that has not been so. The palaeontologist G.G. Simpson was one of the main architects of the 'modern synthesis' that emerged in the 1940s, but his role was to show that the facts of palaeontology were consistent with the mechanisms of natural selection and geographical speciation proposed by the neontologists (a term used by palaeontologists to describe the rest of us), rather than to propose novel mechanisms of his own. Since that time, the attitude of population geneticists to any palaeontologist rash enough to offer a contribution to evolutionary theory has been to tell him to go away and find another fossil, and not to bother the grownups.

In the last ten years, however, this situation has been changed by the work of a group of palaeontologists, of whom Gould has been a leading figure. In his lectures, he stressed two theoretical modifications to the Darwinian scheme — 'punctuated equilibria' and a hierarchical view of evolution. Punctuational theory holds that most evolutionary change occurs when lineages split — that is, when a single species gives rise to two. Between such events, species remain morphologically unchanged, often for millions of years: they remain in 'stasis'. In itself, the theory says nothing about the mechanism of the changes when they do occur, other than that they are rapid in geological terms: they could still be slow to a geneticist, and brought about by the familiar process of natural selection. As J.S. Jones (University College, London) remarked in an earlier discussion, one man's punctuation is another man's gradualism.

Little was said in Cambridge about the empirical evidence for punctuation. Gould treated it as a fact the whole world knows: as something proved by Aunt Jobisca's theorem. As an outsider, I am persuaded that morphological evolution proceeds at very different rates at different times, but can see little evidence that rapid change is necessarily associated with the splitting of lineages (although one would expect this often to be the case, when part of a species enters a new ecological niche). What is new is the emphasis on stasis. As David Wake (University of California, Berkeley) pointed out during the discussion, the existence today of pairs of species which are morpho-

logically indistinguishable, or almost so, but whose proteins have diverged sufficiently to suggest that they have been separate for millions of years, supports the reality of stasis. Stasis, then, is a phenomenon that calls for an explanation.

Geneticists and palaeontologists tend to differ in the explanations they prefer. Population geneticists argue that if a species does not change, this is because it is subject to 'normalizing selection' — that is, selection that eliminates the extreme phenotypes and favours the norm. They have two main reasons for this belief. First, if directional selection is applied to almost any characteristic, in nature or in the laboratory, the population will change. Second, most species with a wide geographical range do vary in structure and behaviour, sometimes to such an extent that populations from different regions would be regarded as different species if they were not connected by a series of intermediate forms. (In case anyone supposes that membership of a species is decided unambiguously by hybridization, it is the case that different species often produce fertile hybrids, and populations of the same 'species' often prove, on investigation, to produce sterile or inviable hybrids.)

In reply, Gould asks how it could possibly be the case that normalizing selection could favour the same phenotypes over long periods, during which the environment may change drastically. Instead, he proposes a concept of species stability arising from developmental constraints. The constancy of a species would thus resemble the constancy of a chemical element: it would represent a stable state of matter, which can be changed only by an unusual event. C.H. Waddington's idea of 'canalization' has been quoted in support of this view. Waddington did indeed point out that the typical 'wild-type' morphology of a species is usually well buffered against disturbance during development, whereas the phenotypes of individuals with a given mutation are highly variable between individuals. However, for Waddington, the uniformity of the wild type was itself the product of normalizing selection, and not a manifestation of an intrinsically stable state.

Hierarchies

The debate about stasis and punctuation will no doubt continue. Gould's second thesis concerned the hierarchical structure of evolution. Darwin, he argued, thought of natural selection as favouring some individual organisms at the expense of others. However, selection, and also chance, can be effective at two other levels, namely the gene and the species. At the level of the

gene, we now know that there are processes (gene conversion, unequal crossing-over, transposition) that enable genes to multiply out of phase with the organisms in which they find themselves. It follows that a gene can increase in frequency by virtue of its ability to multiply horizontally within an individual, and not only by virtue of its effects on the individual's phenotype.

Two points were made about this level of selection during the discussion. John Fincham (Cambridge University) pointed out that since transposons are in effect parasites, we can expect them to evolve 'self-restraint', as some parasites do: it does not pay to kill the goose that will transmit you to future generations. A more fundamental disagreement was raised by Gabriel Dover's (Cambridge University) claims for 'molecular drive' — a portmanteau term for the three processes listed above. I may give an unfair account of this disagreement, because I was one of the protagonists. Dover asserted that there is nothing that natural selection can do that cannot be done by molecular drive. I argued in reply that this is to overlook the fact that natural selection can generate highly improbable adaptations at the organismic level, whereas molecular drive cannot. As I see it, Dover's view would throw out the Darwinian baby with the bathwater.

Species selection

Palaeontologists can contribute little to this debate, but have a lot to say about the level of species selection. The concepts can best be explained by some examples. First, during the Cretaceous, the Volute gastropods gradually replaced their competitors. A plausible explanation is that Volute development did not involve a stage of mobile planktonic larvae, whereas the development of their competitors did. As a result, geographical speciation was more frequent among Volutes, and the number of species increased. In effect, Volute species had a higher 'birth rate'. If this explanation is correct, it applies the concept of natural selection, but replaces individuals by species as the units selected.

Now consider a second example, the mammalian secondary palate. This is a structure that enables a mammal to breathe and chew at the same time. Gould would agree that such a structure (together with associated changes in teeth, jaw muscles and articulation) could arise only by natural selection at the level of individuals. Suppose, however, that mammalian species came into competition with reptiles lacking these adaptations. If the mammals outcompeted the reptiles because they could chew better, then mammalian species would replace reptilian ones. However, Gould would not call this species selection, because the success of the mammals would depend on something individual animals do (chew) and not on something species do: in contrast, in the Volute example, speciation is something species and not individuals do.

* The Tanner Lectures were delivered in Cambridge under the auspices of Clare Hall, 30 April–2 May 1984.

There is a third scenario. For example, at the end of the Cretaceous the Dinosaurs became extinct, whereas the mammals survived and radiated. I do not know why this was so, but let us suppose that the mammals survived because they are homoiotherms, and that their ability to chew had nothing to do with it. It would still be true that the Cretaceous extinctions led to an increase in the proportion of species with secondary palates, but not because of any selection for the palate itself. This is what Elizabeth Vrba has called the 'effect hypothesis'. It is an analogue, at the level of organs, of the process of 'hitch-hiking' at the level of genes.

The hierarchical view of evolution, then, is that processes of selection and stochastic drift go on at the level of genes and of species, as well as of individuals. As an old-fashioned proponent of the modern synthesis, I have no difficulty in accepting this, so long as no one expects me to believe that adaptations of individuals can be explained by molecular drive, and so long as the concept of species selection is confined to qualities, such as speciation rate and evolutionary rate, that are properties of species and not of individuals. However, there remains plenty of room for disagreement about the relative importance of these various levels, or — what amounts to the same thing — about the relative effectiveness of selection at different levels.

In from the cold

This brings me to what I see as the greatest impact that palaeontology is having on the way we see the mechanisms of evolution. We have been familiar for a long time with the dramatic disappearance of the Dinosaurs at the end of the Cretaceous. It is now apparent that massive extinctions, involving many different taxa, have been a repeated feature of evolution. Adolf Seilacher (Tübingen University) and Anthony Hallam (Birmingham University), the two palaeontologists on the panel, agreed with Gould on this, although there was disagreement about whether these events have been periodic or irregular, and whether they are caused by extraterrestrial events (meteorites, asteroids) or by terrestrial ones (Hallam, for example, emphasizes the role of changes in the area of the continental shelf caused by continental drift — see *Nature* 308, 686; 1984). The impact of these extinctions is not random; in any given event, some taxa are more affected than others: Seilacher stressed the need for a quantitative study of this, to replace the somewhat anecdotal picture we now have.

In addition to the problem of their causation (which at present seems to be a problem for geologists and astronomers, although Seilacher did not rule out the possibility that some extinctions had biotic causes), these extinctions raise questions for evolutionary biologists. Is it possible that evolutionary change would slow down and stop in the absence of changes in the physical environment? As Manfred Eigen

has pointed out, the simplest evolving systems (populations of RNA molecules in test tubes) reach a global optimum and then stop. Are extinctions, then, a necessary motive force of evolution? A second question concerns the relation between extinction and radiation. Ecologists tend to see nature as dominated by competition. They would therefore expect the extinction of one species, or group of species, to be caused by competition from another taxon. Most palaeontologists read the fossil record differently. The Dinosaurs, they believe, became extinct for reasons that had little to do with competition from the mammals. Only subsequently did the mammals, which had been

around for as long as the Dinosaurs, radiate to fill the empty space. The same general pattern, they think, has held for other major taxonomic replacements. Not all palaeontologists would agree, but I think this is the majority view. I find it surprising: I would have expected a major cause of extinction to be competition from other taxa.

The Tanner lectures were an entertaining and stimulating occasion. The palaeontologists have too long been missing from the high table. Welcome back. □

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Malarial immunity

Indonesian and Sudanese style

from F.E.G. Cox

RECOVERY from malaria is accompanied by the production of antibodies that block the entry of merozoites — the infective stage of the *Plasmodium* parasite — into red blood cells¹. Alternatively, it can be accompanied by a mechanism that kills the developing parasites within the red blood cell. This phenomenon has been known since 1944, when the Taliaferros noted the appearance of disintegrating forms, 'crisis forms', of a simian malaria parasite, *Plasmodium brasilianum*, in the red cells of recovering monkeys², but received little attention until it became clear that crisis forms are associated with recovery from a number of different *Plasmodium* infections in laboratory animals³. Although crisis forms are not apparent in the peripheral blood of patients with malignant tertian malaria caused by *Plasmodium falciparum* (because the developing stages of the parasite disappear from the circulating blood), they can regularly be detected *in vitro* both visually and by a test in which the incorporation of radiolabelled hypoxanthine into growing parasites within the red cell is measured⁴. That test has recently enabled James Jensen and his colleagues to show that in the Sudan, and probably elsewhere, intraerythrocyte killing predominates over merozoite blocking as a means of defense against the parasite⁵⁻⁷. These observations should completely alter our concepts of the development of vaccines against the blood-borne forms of *Plasmodium falciparum*.

Jensen and his colleagues collected sera from some 300 people living in three regions of the Sudan and tested their ability both to block the invasion of red cells by merozoites and to induce crisis forms in erythrocytes containing 'ring stage' parasites. The population could be divided into those who had experience of serious attacks of malaria, of mild malaria or no experience of malaria⁶. The clinical status could not be correlated with the presence of

merozoite-blocking antibodies but was correlated with the ability of the serum to induce crisis forms. The greatest number of crisis forms was induced by sera from the group with no experience of malaria and hence with the greatest immunity to the disease. The serum factor that causes parasites to die in the red blood cells is not antibody because antibodies on the merozoites are sloughed off as the parasites enter the red cells; moreover, activity is not lost with the removal of immunoglobulin⁵. Jensen and his colleagues call the factor 'crisis-form factor' (CFF)⁶.

In the Sudan, then, clinical immunity to malaria can be correlated with the presence of CFF in the serum. But what of other parts of the world? Jensen *et al.* have now shown that Indonesian sera do not induce crisis forms *in vitro*⁷. In order to make a direct comparison, the earlier Sudanese studies were repeated with sera from an area where the prevalence of malaria was similar to that in Flores, where the Indonesian study was carried out. Overall, Sudanese sera have abundant CFF and little merozoite-blocking antibody whereas Indonesian sera lack CFF, and their anti-parasitic activity, where present, is associated with merozoite-blocking antibody. This indicates that genetic differences may be important in determining whether antibody or non-antibody factors predominate in immunity to human malaria.

These experiments are of great importance and should significantly alter our concepts of an antimalarial vaccine. It is conventional to identify and isolate blood-stage antigens of the parasite by means of monoclonal antibodies and immune sera⁸. Such techniques may well lead to a vaccine that elicits a high and specific antibody response. But will this be protective when sera with antibody titres greater than 1:10,000 have little merozoite-blocking effect⁷? And if merozoite-blocking is of

relatively little importance in natural immunity in Sudan, could it be the basis of an effective vaccine there? If genetic differences between the populations at risk have to be taken into account in malaria vaccine development, it will greatly complicate the task.

What is CFF? The short answer is that we do not know. As long ago as 1953, Trager and McGhee identified a factor in the sera of birds immune to malaria that inhibited infection by Rous sarcoma virus⁹. From this has arisen the possibility that CFF is related to the ill-defined tumour necrosis factor^{10,11}. Alternatively, it may be related to certain reactive oxygen intermediates that bring about the occurrence of crisis forms both *in vivo* and *in vitro*^{12,13}. However reactive oxygen intermediates are probably too short-lived to produce directly the effects recorded by Jensen *et al.*, although they could be involved in reactions with lipids to produce toxic substances such as aldehydes¹⁴.

What must happen now is a switch of attention away from *Plasmodium* antigens that elicit the production of antibodies to those that elicit CFF. Since CFF is probably a product of macrophages, these cells and the T cells that activate them require much more attention. At a very basic level, however, we now know that CFF kills malaria parasites and thus, if characterized, could be used to treat the rapidly increasing number of drug-resistant cases for which there is no obvious alternative treatment. Further studies of CFF might also provide clues to the development of new antimalarial drugs. □

1. Cohen, S. & Butcher, G.A. *Immunology* **19**, 369 (1970).
2. Taliaferro, W.H. & Taliaferro, L.G. *J. infect. Dis.* **75**, 1 (1944).
3. Clark, I.A., Cox, F.E.G. & Allison, A.C. *Parasitology* **74**, 9 (1977).
4. Clark, I.A. *et al. Lancet* **i**, 359 (1983).
5. Jensen, J.B., Boland, M.T. & Akood, M. *Science* **216**, 1230 (1982).
6. Jensen, J.B. *et al. Infect. Immun.* **41**, 1302 (1983).
7. Jensen, J.B. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 922 (1984).
8. Coppel, R.L. *et al. Nature* **306**, 751 (1983).
9. Trager, W. & McGhee, R.B. *Proc. Soc. exp. Biol. Med.* **83**, 349 (1953).
10. Clark, I.A. *et al. Infect. Immun.* **32**, 1058 (1981).
11. Taverne, J., Dockrell, H.M. & Playfair, J.H.L. *Infect. Immun.* **33**, 83 (1981).
12. Clark, I.A. & Hunt, N.H. *Infect. Immun.* **39**, 1 (1983).
13. Ockenhouse, C.F. & Shear, H.L. *J. Immun.* **132**, 424 (1984).
14. Ferrante, A., Rzepczyk, C.M. & Allison, A.C. *Trans. R. Soc. trop. Med. Hyg.* **77**, 789 (1983).

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100 years ago

THE *Bolletino* of the Italian Geographical Society for May contains a brief account of Signor Maurizio Buonfanti's late expedition across North Africa. After some trips to Yakoba and other little-known parts of Sokoto, he made his way through Gando to the Niger at Say, about midway between Timbuktu and the Binue confluence. Here he turned north, and for the first time ascended the Niger as far as Timbuktu. This feat, hitherto supposed to be impossible, was performed in the dry season, and the problem thus successfully solved possesses considerable geographical and commercial importance in connection with the attempts now being made to establish regular lines of water communication between Western and Central Sudan and the Gulf of Guinea. From *Nature* **30**, 131, 5 June 1884.

Astronomy

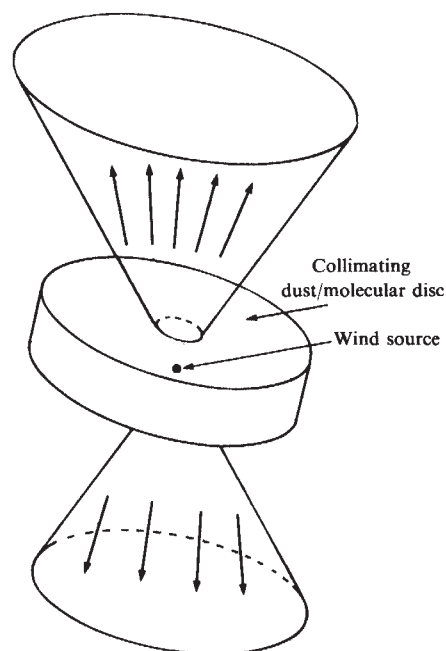
High-velocity winds in Orion

from Ben Zuckerman

THE biggest surprise to emerge from a decade of observational study of the formation of stars has been the realization that this process is accompanied by massive outflowing winds. The winds, which can be sporadic, are millions of times stronger than the solar wind and are generated by most, perhaps all, young stars either shortly before or, more probably, shortly after they initiate the burning of hydrogen into helium in their interior. Since it is natural to think of star formation as a process of collapse, or infall, the ubiquitous occurrence of an accompanying outflow comes as a bit of a shock. Now D.J. Axon and K. Taylor have described new optical spectra indicating very high-velocity winds in the most famous region of star formation in the heavens, the Orion Nebula (*Mon. Not. R. astr. Soc.* **207**, 241; 1984). Their spectra point towards the existence of a biconical pattern of outflow and suggest the presence of a substantial disc of molecular gas and dust grains close to a putative stellar source of excitation, located in the giant molecular cloud behind the nebula.

As a result of isolating the 6,300 Å wavelength forbidden transition of neutral oxygen, which is prominent in shock-excited regions but relatively weak in photoionized gaseous nebulae such as the Orion Nebula, Axon and Taylor discovered a 'family' of six shock-excited objects which, in the plane of the sky, are located near a group of strong infrared-continuum sources discovered in the 1960s. The latter are believed to constitute a very young, but highly obscured, star cluster behind the Orion Nebula. The oxygen atoms display a very wide range of line-of-sight velocities extending from near 0 km s⁻¹ (the velocity of the molecular cloud) to an approach velocity of nearly 400 km s⁻¹. When projection and other effects are taken into account, the implied wind velocities are of the order of 1,000 km s⁻¹ — similar to that of winds from the most massive main-sequence stars in our Galaxy.

Taking into account previous observations of Orion, primarily at infrared and microwave frequencies, Axon and Taylor conclude that the most plausible model of the region is one in which a biconical protostellar wind originates from one (or more) of the massive young stars that are embedded in the molecular cloud, as in the figure. Although the star itself must be obscured from our view by an optically thick disc that lies, presumably, in its equatorial plane, the wind can escape from the polar regions. In their model, the shock-excited objects, detected by neutral oxygen emissions, are the result of the impact between the winds and gas blobs ('knots') located at the front face of the molecular cloud, just



Model of biconical protostellar wind in the Orion Nebula. (From *Mon. Not. R. astr. Soc.* **207**, 246; 1984.)

behind the Orion Nebula itself.

These results bear on more general questions related to star formation and massive winds. Observations of many other regions of star formation indicate that bipolar or biconical outflow is the rule rather than the exception. Orion has now been reasonably securely added to this class. If the collimation is due to a disc of gas and dust located near the stars, as Axon and Taylor, like many others before them, suggest, then it is possible that at least the raw material necessary to make planets can be found in the vicinity of most young stars. Since not a single planet has been identified with certainty outside our Solar System, the implied ubiquitous existence of this raw material is of considerable interest.

Material which may be distributed in the form of a small disc with dimensions only about three times that of our Solar System has very recently been seen directly (via scattered light at 2 μm wavelength) near the very young star, HL Tauri, by two different teams (S. Beckwith, B. Zuckerman, M. Skrutskie and H.M. Dyck, and S. Strom and G. Grasdalen, *Astrophys. J.*, in the press). However, it still remains to be shown how frequently a gaseous disc, as opposed to some other physical mechanism such as a magnetic field, is actually responsible for collimation of the winds that accompany star formation. □

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Protein structure

From *Bacillus* to rabbit

from Roger H. Pain

ON page 467 of this issue, Poorman *et al.* suggest a three-dimensional structure, complete with binding sites for substrates, activators and inhibitors, for a mammalian multisubunit enzyme, largely on the basis of the amino acid sequence of the enzyme and the crystallographic structure of the equivalent, but smaller, enzyme from a bacterium. Their report is of intrinsic interest to the protein chemist since it suggests a model which is testable by experiment. It is also of broader interest because it illustrates both the confidence with which biologists now assert that protein conformation is selected for and preserved during evolutionary processes and the way in which speculation on protein structure can be in order.

The enzyme is phosphofructokinase (PFK). It operates in the glycolytic pathway which is the means, common to all living organisms, of abstracting useful energy from the oxidation of glucose. PFK is particularly important because its activity can be modulated to control the rate of oxidation of glucose in response to energy demand in the cell. The reaction catalysed is the phosphorylation of fructose 6-phosphate by ATP to yield fructose 1,6-diphosphate and ADP. Insofar as they are all activated by raised concentrations of ADP, the enzymes from all species behave similarly. The greater sophistication of higher organisms, however, is reflected by the fact that their PFK is also activated by fructose 1,6-diphosphate and is inhibited by ATP. Moreover, their PFK is inhibited by citrate, allowing feedback control from the citric acid cycle. The greater complexity of the eukaryotic enzyme, requiring additional specific binding sites, is reflected in a molecular weight for each of its four identical subunits that is approximately double that of the prokaryotic enzyme.

To date, the only three-dimensional structure of PFK to have been determined is that of the prokaryote, *Bacillus stearothermophilus*. The structure of a eukaryotic PFK, the rabbit enzyme being the most studied, is needed in order to understand the more sophisticated mechanisms by which it is controlled. Poorman and colleagues have attempted to provide this, based mainly on comparison of amino acid sequences. They have determined the sequence of rabbit PFK and found that the subunit sequence falls into two similar halves, with more than 30 per cent of their amino acids being identical. Comparison of the two halves with the sequence of *B. stearothermophilus* PFK shows that 34 per cent of the amino acids of the carboxy-terminal half, and 44 per cent of the amino-terminal half, of the rabbit

enzyme are identical to those in the bacterial enzyme.

This is taken to signify that the eukaryotic enzyme is the result of a duplication of the prokaryotic gene, with subsequent independent mutation within certain constraints. A further unknown mechanism must account for the additional 30 amino acids which join the non-identical twin domains of the rabbit enzyme. By packing four such 'double-bacillus' subunits together, with due regard to symmetry and the location of ligand-binding sites, Poorman *et al.* have constructed their three-dimensional model of rabbit PFK.

By straightforward extrapolation from the bacterial enzyme, rabbit PFK would be expected to contain 8 substrate binding sites and 8 ADP effector sites. Sequence comparison has led to identification of the four fructose 6-phosphate binding sites that are known to exist in rabbit PFK; sequences for the other four are significantly altered by mutation and are postulated to be fructose 1,6-diphosphate activation sites. It is also assumed that four ADP effector sites have been mutated to

form ATP inhibitory sites.

These arguments rest on two generalizations. First, that such close relationships between amino acid sequences are the result of the organisms being at points on an evolutionary tree separated by a divergent process. Second, it is assumed that despite appreciable changes in amino acid sequence and despite the fact that certain amino acid substitutions can lead to a dramatic destabilization of three-dimensional conformation, the two sequences will fold into similar conformations. The 'code' relating sequence to conformation is known to be degenerate. The assumption is that selection operates on the one hand to keep the changes in sequence within these limits of degeneracy so that conformation does not vary significantly and, on the other hand, to retain certain active sites and to allow others to be modified so as to lead to more advantageous catalytic activity. This emphasis on the conservation of conformation is intriguing and the reasons for it are by no means certain. It is fascinating to see how these generalizations, only relatively recently established by comparing known sequences and conformations, are now being used in reverse to predict conformations from sequence — in this instance, of rabbit PFK. □

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Bacterial motion

Progress in flagellation

from Michael Spencer

IT is now more than ten years since the electrifying announcement that bacterial flagella actually rotate rather than propagate helical waves like larger motile organelles. The discovery was all the more surprising because it was by then becoming clear that flagella could adopt a number of different waveforms by internal rearrangement of the flagellin monomers, and an extension of this to a mechanism of active wave propagation did not require any dramatic act of faith.

From that watershed in knowledge have flowed two quite separate streams of thought. From recent publications, however, it now looks as if these independent streams, which have followed their courses for so long without meeting, are on the point of converging. Prominent in one stream is the school of Sho Asakura, which for at least two decades has continued a series of elegant demonstrations of the polymorphic nature of flagellar structure. These studies have been complemented by the ingenious theoretical predictions of a structural engineer, C.R. Calladine, and have led to satisfying explanations of the transitions that enable bacteria to change direction. The other stream of research has

concentrated on the morphology and dynamics of the basal body that constitutes the flagellar motor; this has involved groups led by Howard C. Berg and others. They have confirmed that the motor is powered not directly by ATP but by protons, so that an electrical potential or a pH gradient across the membrane is sufficient to generate rotation.

Interest has been growing recently in the short region linking the basal body to the flagellum; it is normally sharply curved (and called the 'hook') and helps the larger-scale helix of the flagellar filament to insert into the motor in a direction perpendicular to the cell wall. It may also have enough flexibility to allow flagella to group into bundles. The structure of isolated hooks was determined a few years ago by Wagenknecht *et al.* (*J. molec. Biol.* **151**, 439; 1981 and **162**, 69; 1982). Although the monomers form a tubular structure and are packed in a very similar way to those in flagella, there are deep grooves in the surface of the hook that could facilitate bending without unduly reducing the ability to transmit a torque.

Some interesting new findings have now been published by Kato, Okamoto and

Asakura (*J. molec. Biol.* **173**, 463; 1984). They studied a mutant hook, the 'poly-hook', which is much longer than in the wild type and can adopt either a straight form or a helical one; in the latter the amplitude and pitch are much smaller than those of the flagellar filament. Kato *et al.* now have evidence for both left-handed and right-handed helices, and suggest that the hook undergoes transitions between helical senses in the same way as the flagellar filament.

Like many discoveries, this raises more questions than it answers. The first surprise is that the 'normal' form of the hook (at neutral pH) follows a right-handed helix, whereas the rest of the flagellum is left-handed in the species studied — *Escherichia coli* and *Salmonella typhimurium*. This means that when the motor starts up in its normal sense of rotation, which is counterclockwise when looking towards the base, the torque transmitted to the hook is such as to unwind its helix (readers who feel dizzy when trying to visualize this are referred to Fig. 1a). In these conditions flagella tend to flip into an alternative helix of opposite sense.

Kato *et al.* speculate that when the motor starts up, the resulting stress may transform the hook into the alternative left-handed type, which would then have the same sense as the main flagellar filament. This raises intriguing questions about what happens during 'tumbling' or 'twiddling', the process by which bacteria periodically change direction so as to move up a favourable gradient (for a review see Berg *Scient. Am.* **233**, 36; 1975). At the start of a tumble the direction of motor rotation reverses and bundles of flagella that previously rotated in phase become jammed; the stress induces a change of sense in the flagella which then fly apart (Macnab and Ornston *J. molec. Biol.* **112**, 1; 1977). If the hook is held in a left-handed form during normal motion, presumably it reverts to right-handed when the motor reverses. Is this an essential step in the transformation of the filament?

A second question raised by the new results is whether the hook transformation can be explained on the model proposed for flagella by Calladine (*J. theor. Biol.* **57**, 469; 1976 and *J. molec. Biol.* **118**, 457; 1978). This model presumes the existence of 'protofilaments' or strings of monomers which may (through cooperative changes in monomer conformation) adopt either a 'long' or a 'short' state. If the protofilaments run round the tubular structure at an angle to its axis, the existence of two classes of protofilament in the same flagellum will cause it to coil into a helix (Fig. 1b). The parameters of the helix depend on the relative population of the two states.

The model predicts that in going from one state to another the 'twist' of the protofilaments will change, and that between a left-handed and a right-handed helix the 'twist angle' changes sign. In flagella the twist angle varies between -3° and $+8^\circ$,

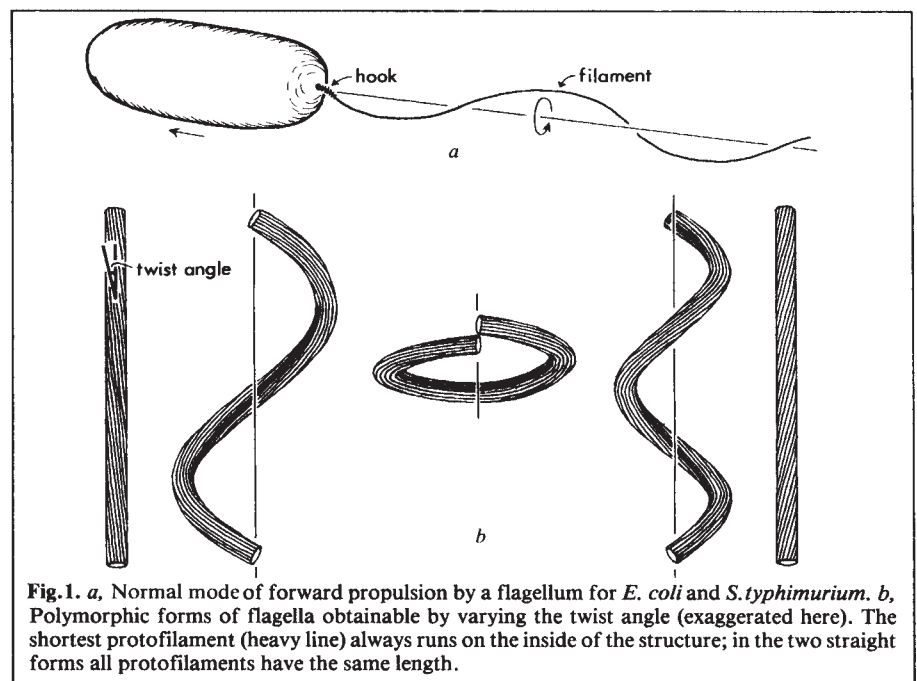


Fig. 1. a, Normal mode of forward propulsion by a flagellum for *E. coli* and *S. typhimurium*. b, Polymorphic forms of flagella obtainable by varying the twist angle (exaggerated here). The shortest protofilament (heavy line) always runs on the inside of the structure; in the two straight forms all protofilaments have the same length.

which does not demand a substantial change in monomer packing. In the poly-hook, however, the observed helical waveforms (from which twist angle was obtained indirectly) would require a range of about -50° to $+20^\circ$. Even more puzzling, the protofilaments appear to be identified not with the 11 rows of contiguous monomers that fulfil this function in the flagellar filament, but with a set of '16-start' helices joining monomers that are not in close contact with each other.

Kato *et al.* are suitably cautious about this conclusion, and mention the possibility that preparation for microscopy may have deformed the polyhooks; the matter will have to await direct observation of twist angles by high-resolution imaging of the surface lattice. A possibility not discussed in the paper is that in the hook the basic structure is invariable, as suggested by Wagenknecht *et al.* (see above), and that left-handed and right-handed forms involve protofilaments joining two different sets of points in the helical net. Neither of these needs necessarily be the set of 16-start helices discussed by the authors.

By a curious coincidence, a paper by Block and Berg on page 470 of this issue gives fresh significance to the old observation that certain components of the basal body 'motor' have a 16-fold symmetry. These authors have been studying a mutant of *E. coli* whose flagella are intact but paralysed unless the missing gene product is supplied. The appropriate gene under the control of the *lac* promoter was arranged to be carried into the bacteria as part of a transducing phage; the gene product was synthesized on addition of a *lac* inducer. When carried out with bacteria tethered to a glass surface by a single flagellum, the cells began to spin with increasing speed a few minutes after the addition of inducer.

The most interesting feature of the results is that the speed of rotation

increased (or occasionally decreased) in discrete steps; at first there was a doubling of speed, which was followed by increases to speeds that were all multiples of the initial value for a given cell. The initial speed varied from cell to cell, presumably because of variations in the viscous drag on the cell body. Discrete steps could not be resolved beyond about the sixth, and most cells came off the glass or stuck to it before reaching their terminal speed. However, from one cell that continued rotating up to maximum speed and from other experiments on wild-type cells, Block and Berg calculate that the maximum number of steps is close to the magic number of 16. Their interpretation of this behaviour is that the gene product released by the *lac* inducer diffuses to the vicinity of the flagellar motor, which contains a number of independent force-generating units each requiring the gene product.

Block and Berg calculate that about 65 protons pass through a single force generator in one revolution, if the efficiency of the machine is unity — an assumption that may raise eyebrows among muscle biochemists. What might also attract their attention is that the torque per generator is calculated to be about 3×10^{-12} dyne cm; assuming that the generators act at a radius of 10 nm, one can infer that the tangential force per generator is about 20 times the average force generated by a muscle cross-bridge. In that sense bacteria seem to have a better design than muscle, and workers on flagella may have the more tractable problem. They still, however, share with devotees of muscle the need to find out how force is generated at the molecular level. □

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Neurology

Hopes of central repair

from Jeffrey S. Nye

THE fact that the adult central nervous system cannot be repaired after injury and that neurological deficits are permanent and unchanging was challenged recently at a conference* which brought together clinical and basic neuroscientists who are studying regenerative and repair processes in the central nervous system. The meeting made clear that clues from animal models of degenerative disease, as well as basic studies of regeneration in the nervous system, are increasing our understanding of neurodegenerative disease. Moreover, a radical change in the way some of the diseases are treated may be in the offing.

Controversy continues about the extent and importance of neurogenesis in adulthood¹. Classically, only during embryonic life do neuronal precursor cells multiply. They then migrate from the ependymal or subependymal zones to their ultimate destination, and only there differentiate into neurones or glia. In fish and birds, however, the evidence for neurogenesis in adulthood is clear. Fernando Nottebohm (Rockefeller University) has shown that some avian species destroy and regenerate each year the neurones in certain forebrain nuclei². In these nuclei, which subserve song production and perception, the acquisition and loss of neurones precede the bird's yearly acquisition and loss of song³. Thus neurogenesis serves an adaptive function in the life of songbirds. In the dentate gyrus and the olfactory bulb of adult rats, select populations of cells are born which are clearly neurones and not glia, in view of the presence of synapses and neurochemical markers^{4,5}.

Neuronal plasticity does not depend exclusively on the formation of new neurones. In primates, for example, all neurogenesis is complete soon after birth, but learning continues throughout adulthood. Pasko Rakic and colleagues (Yale University) argue that each neuronal population is born within its own discrete span of time during embryogenesis, some continuing into early infancy⁶. Despite extensive searching, no neurones born after this period have been found in primates, with the sole exception of the olfactory neurones, which are renewed throughout the lifetime of the organism⁷. Restoration of the capacity for neurogenesis in adult primates continues to be an attractive challenge to many neuroscientists.

Instead of growing new neurones, adult primates adapt by forming new synapses (synaptogenesis) and modifying old ones. What stimulates these adaptive processes

remains an open question. In a model system using rats, Carl Cotman has shown that damage to neurones innervating the hippocampus will stimulate surviving neurones to increase the number of synapses they form with the hippocampus⁸. This reactive synaptogenesis may also be important for recovery of function after damage to neurones in aging and in neurodegenerative disease. In the aging rat, the rate of synaptogenesis after injury slows, but the same final number of synapses is reached as in younger animals. Quantitative similarity in the number of synapses, however, does not explain well known differences between the responses of old and young rats to brain damage. Indeed, Cotman has found decreases in the number of synapses contralateral to the injury in the hippocampus of old rats. He also finds differences in glial secretion of neuronal trophic factors in old versus young rats⁹. These findings may offer a possible mechanism for the poor recovery of old animals after injury.

A highlight of the meeting was the presentation by three groups of the startling results of their studies of neural tissue grafts. Albert Aguayo (Montreal General Hospital) and his group have been studying axonal growth after injury to the central nervous system¹⁰. In contrast to peripheral nerves, central neurones do not regrow axons past an injury; normally, they are prevented from doing so by astrocytes and oligodendroglia. However, when peripheral nerve sheaths, containing Schwann cells, are grafted next to the site of such an injury, some central neurones will extend axons as far as 3 cm through them. But when they reach the end of the peripheral nerve graft and re-enter damaged central nervous system, they stop growing and make no functional connections.

Unfortunately, this impediment must be overcome before grafts of peripheral nerve can be used to repair damaged central nervous tissues.

Another approach to restore function after injury or in neurodegenerative disease requires grafts of neurones similar to those which are damaged or missing. Pioneering work has demonstrated that intracerebral grafts will establish reciprocal synapses with surrounding nervous tissue¹¹. Functional recovery, however, requires that the grafted neurones establish a network of connectivity similar to that found in undamaged brain. The stringency of this requirement may depend on the site of damage. Might fetal cells, which are developmentally ready to send out processes and establish connections, retain these abilities outside their normal environment?

In a number of successful experiments, Lars Olson and colleagues (Karolinska Institute) have demonstrated that fetal neurones will survive grafting into the anterior chamber of the eye of a rat¹². Moreover, neurones from the fetal substantia nigra implanted into the eye will generate the same firing pattern that they do *in situ*. When implanted adjacent to a fragment of striatum derived from an adult rat, fetal nigral cells innervate and establish functional connections with it.

In a similar series of experiments, the Lund group, led by Anders Bjorklund, has shown that injected fetal neurones from the septal nuclei will innervate a denervated hippocampus¹³. The implanted cells elaborate a pattern of cholinergic projections remarkably similar to that of the intact hippocampus. Even more astonishing, the grafts enable rats that have lost their cognitive abilities in a T-maze to regain them. Although the injected septal cells could not have received afferents from normal sources innervating the septum, the cells apparently possess enough intrinsic information to restore function to a denervated hippocampus.

Interestingly, Olson has also demonstrated that adult adrenal chromaffin cells can substitute for substantia nigra in innervating intraocular striatum¹³. In animal models, the implanted chromaffin cells begin to secrete dopamine, instead of their usual product, adrenaline. Both of the European groups have used dopaminergic cells from fetal substantia nigra to alleviate the motor deficits in animal models of parkinsonism. Furthermore, Olson has extended his studies of adrenal medullary implants, and shown that they too will provide enough dopamine to prevent some of the extrapyramidal symptoms of parkinsonian rats. For application to human disease, the use of adrenal grafts would avoid the ethical and immunological problems of using cells from fetal donors.

Olson's group has proceeded stereotactically to graft adrenal tissue from two patients with severe medically intractable

- Altman, J. *Science* **135**, 1127 (1962).
- Goldman, S.A. & Nottebohm, F. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2390 (1983).
- Nottebohm, F. *et al. Brain Res.* **213**, 99 (1981).
- Bayer, S.A. *Expl Brain Res.* **50**, 329 (1983).
- Kaplan, M.S. & Bell, D.H. *Expl Brain Res.* **52**, 1 (1983).
- Rakic, P. *Neurosci. Res. Prog. Bull.* **20**, 439 (1982).
- Graziadei, P.P.C. & Monti-Graziadei, G.A. in *Handbook of Sensory Physiology* Vol.9 (ed. Jacobson, M.) (Springer, Berlin, 1978).
- Cotman, C. & Nieto-Sampedro, M. *A. Rev. Psychol.* **33**, 371 (1982).
- Nieto-Sampedro, M. *et al. J. Neurosci.* **3**, 2219 (1983).
- Aguayo, A.J. *et al. in Advances in Cellular Neurobiology* Vol.3 (eds Feteroff, S. & Hertz, L.) (Academic, New York, 1982).
- Bjorklund, A. & Stenevi, U. *A. Rev. Neurosci.* **7**, 279 (1984).
- Olson, L. *et al. in Neuronal Transplants, Development and Function* (eds Sladek & Gash) (Plenum, New York, in the press).
- Bjorklund, A. *et al. Acta physiol. scand. Suppl.* **522**, 1 (1983).
- Olson, L. in *Synaptic Plasticity and Remodeling* (ed. Cotman, C.) (in the press).
- Crutcher, M.D. & DeLong, M.R. *Expl Brain Res.* **53**, 233 (1984).

*The fourth annual conference of the Institute for Child Development entitled 'Hope for a new neurology' was held in New York City on 16-18 April.

Parkinson's disease into the head of the caudate of the brain¹⁴. The success has been marginal. One patient showed no improvement (and no additional impairment) and the other has had some minor reduction of symptoms in the arms alone. Olson speculates that the caudate might not have been the right location for the graft to prevent motor symptoms; recent studies on monkeys have suggested that the putamen is the striatal site more closely correlated with motor function¹⁵.

Despite these initial clinical failures, information from animal studies of neural grafting is accumulating rapidly. As work on the pathophysiology of Parkinson's and other neurodegenerative disorders progresses, investigators will test their new animal models with intracerebral grafts. It is likely that within a decade or two, neurologists will have another therapeutic option for severely disabled patients, and a new neurology. □

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Cosmology

Coloured comets

from David W. Hughes

WHAT colour is a comet? There is no easy answer. Visually, they are rarely seen in true colour because the retinal cones, which respond to colour, are usually swamped by the rods when it comes to the nocturnal vision of faint objects. George F. Chambers, a prolific cometary observer at the turn of the century, found that comets 'exhibit a more or less silvery-grey hue'. There were some exceptions. The sensational assertions of our mediaeval ancestors that comets had the colour of blood, or were fiery red, were discounted by Chambers, although tinges such as yellow, yellowish and ruddy were thought possible, particularly for bright comets.

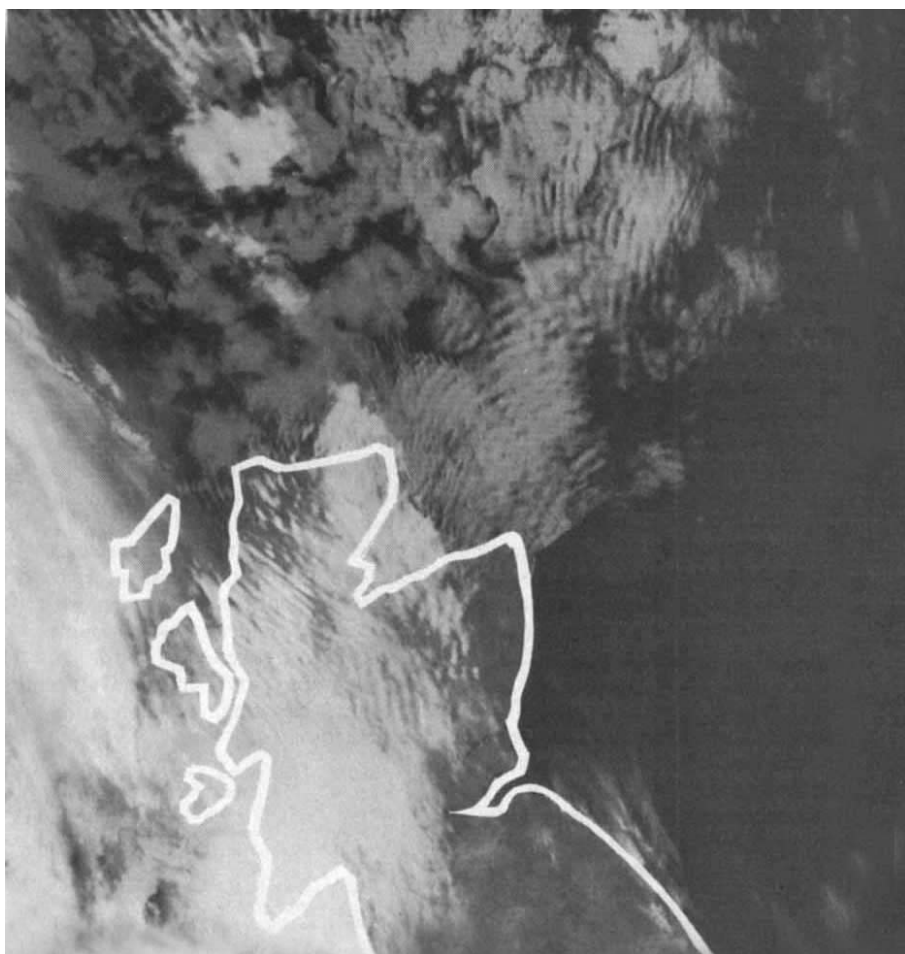
A quantitative approach to cometary colour can be made by measuring their brightness through a set of filters, as used recently by William Hartmann and Dale Cruickshank (*Icarus* 57, 55; 1984). They analysed the V, J, H and K brightnesses of

fourteen comets through filters centred on 0.55, 1.25, 1.58 and 2.2 μm . Defining an ' α index' as the position of a Solar System object on the J-H-K colour-colour diagram, Hartmann and Cruickshank find, crudely, that low (J-H), (H-K) brightness values (obtained by subtraction) are equivalent to high α s and vice versa. By looking at a range of asteroids, satellites and laboratory substances, the authors find that the α index increases both as a function of increasing albedo and as the ratio of ice to dirt area of the reflecting surface increases. The α index is also a function of colour, low values coming from dirtier redder surfaces, high values from bluer icier surfaces.

Data from the fourteen comets show clearly that cometary α indices increase as a function of heliocentric distance. Put simply, this means that as comets approach the Sun they become brighter and redden.

Unfortunately, the physical effects responsible for the α change are rather convoluted. First, changing the particle size distribution can change the colour, with the introduction of relatively more small particles causing reddening. Second, compositional changes can also affect the colour. Remote comets (such as Schwassman-Wachmann 1 at 6 AU) can release relatively stable icy-dust grains. When the comets are about 1 AU from the Sun, the ice component of the coma grain sublimates within less than an hour, so only the reddish carbonaceous dust remains to reflect sunlight. Notice that this mechanism could explain why the eruption of jets of fresh material from the nucleus can lead to sporadic colour changes — an injection of icier material turning the coma bluer. The third factor is phase. Asteroids certainly redden as they are observed at larger phase angles and comets could follow suit. Comets observed at similar phase angles do, however, exhibit significant differences in colour; the nature of the reflecting and scattering particles must therefore differ.

Hartmann and Cruickshank conclude that comets get redder as they approach the Sun and that this is not just due to a change of phase. The underlying cause is probably tied up with a decrease in the iciness of the dust being pushed away from the nucleus by momentum transfer from the sublimating gases. The closer a comet is to the Sun the hotter this dust becomes. Unfortunately we might have to wait for another brilliant daylight comet, the last of which was 1910 I, before we can see a blood-red comet with the naked eye. □



Infrared image of cloud structures off the north of Scotland, taken by the NOAA 5 satellite at 0335 GMT on 23 March 1982. The wave-like features are caused by buoyancy oscillations — 'gravity waves' — occurring in Arctic or polar maritime air masses. From a recent paper in *Meteorological Magazine* (113, 97; 1984) in which K.J. Weston relates oscillations in wind speed and direction measured at the surface, with periods of about 20 minutes, to such high-altitude phenomena. Photograph: University of Dundee.

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X-ray crystallography

Advantages of electronic 'film'

from Stephen C. Harrison

By comparison with other areas of molecular biology, the determination of macromolecular structure has suffered because of the time-consuming and tedious course of X-ray crystallographic investigations. Recent advances in genetic manipulation have made it possible to produce considerable quantities of all sorts of interesting proteins and protein-nucleic acid complexes, some of which have been crystallized. It is therefore of more than casual interest that as a result of progress in the technology of X-ray detectors we appear to be on the verge of achieving a substantial acceleration in the collection of intensity data from crystals. The new detectors are two-dimensional position-sensitive devices which permit the simultaneous recording of large numbers of reflections with the accuracy usually associated with one-reflection-at-a-time diffractometers.

The earliest X-ray intensity measurements from crystals were made by following ionization with a gold-leaf electrometer. The hands of Bragg *père* and Bragg *fils*, actuated by the beat of a metronome, served as the 'stepping motors' for moving the crystal through its reflecting position. (Bragg junior wrote an entertaining account¹ of the times in which the intensity of the 100 reflection from NaCl could be reported² to be '676, when the crystal was turned through five minutes of arc for each beat of the clock'). As more complicated structures came to be studied, recording with photographic emulsions became standard, since it enabled many reflections to be measured simultaneously. The advent of automated diffractometers in the late 1950s turned a large part of the crystallographic community to electronic data collection, using a proportional counter or similar device, but the inefficiency of recording only one reflection at a time made diffractometry a poor choice for larger or more complicated proteins. A simple calculation presented by Arndt³ shows that for unit cells larger than about 150 Å, appropriate photographic recording is the significantly more efficient approach. Indeed, stepped oscillation or rotation photography has become standard in most laboratories working on large proteins and at synchrotron installations supporting macromolecular crystallography.

Photographic film is actually an excellent detector, and for 1.5 Å X rays it is about 70 per cent quantum efficient. Automated two-dimensional densitometers, such as the widely used rotating-drum scanners, make digitization of films and evaluation of intensities relatively straightforward, although in a number of cases the tedium of this step has proved rate-limiting. A more significant disadvantage of photographic

measurement is the limitation to its accuracy imposed by the small fraction of recording time during which a given reflection is diffracting. In oscillation photography, a 1° photograph is 'typical', but the angular range over which a particular order diffracts might be 0.1–0.2°, depending on geometrical factors and crystal quality. Background is recorded on the film during the remaining 0.8–0.9°, decreasing the signal-to-noise ratio. This disadvantage could be eliminated by the expedient of recording a series of 0.1° photographs — and it is precisely this possibility, impractical with actual film, that is offered by the new devices.

Currently there are three quite different two-dimensional detectors for single-crystal X-ray diffraction, each based on different design ideas and on significantly different technologies. The device developed by U. Arndt at the Medical Research Council Molecular Biology Laboratory in Cambridge, and now marketed by Enraf Nonius, records the diffraction pattern on a fluorescent screen connected by fibre optics to an image intensifier^{4,5}. The intensifier output is read by a television system linked to a computer. The Gd₂O₂S input screen is 47 × 63 mm and the final scan is binned at 512 × 512 pixels. The size of the screen and the point-to-point resolution imply that data to 3 Å resolution can be collected at a single detector setting from crystals with a 100 Å cell constant. Crystals with larger unit cells require a longer crystal-detector distance and several detector settings. The device includes a 4-circle goniostat to facilitate resetting.

The detector developed by N. Xuong and colleagues at the University of California at San Diego is a multiwire proportional counter, derived from detectors developed for high-energy physics^{6,7}. It consists of a xenon-filled ionization chamber with delay-line readout from planes of parallel wires in *x* and *y*. It has an active area of 27 × 30 cm, with a spatial resolution of 0.6 mm full width at half maximum (FWHM) in the horizontal direction and 2 mm in the vertical direction. The modest resolution necessitates a large crystal-detector distance (1 m or more), and full recording of a protein diffraction pattern to 3 Å resolution may require multiple settings or an array of more than one detector. The set-up in Xuong's laboratory has already been used to solve several structures, including dihydrofolate reductase⁸ and the large ('Klenow') fragment of *Escherichia coli* DNA polymerase I (T. Steitz, personal communication). A similar device has been constructed at the University of Virginia.

A different type of xenon-filled detector has been developed by R. Burns of the Xenotronics Corporation, Cambridge, Massachusetts. It is also a multiwire device, but with capacitive readout and with much higher spatial resolution (0.2 mm FWHM in both *x* and *y*). Its active area is 12 cm in diameter, and it is fitted with a modified oscillation 'camera'. The high spatial resolution permits a single detector to mimic a conventional X-ray film. The Xenotronics detector has been used to collect data from several quite challenging crystals, including tomato bushy stunt virus, with a unit cell constant of 383 Å, and in our preliminary experience has shown very favourable intensity statistics⁹.

Each of these devices is likely to have its own niche in the ecology of crystallographic applications. An advantage of the television-based design is its inherently higher data-rate capacity. The xenon-filled devices are at present limited by dead time to a total rate (over the face of the detector) of 30–50 kHz. Although not a significant disadvantage with conventional sources, this limitation can restrict possible applications with synchrotron sources. The Xenotronics device has the advantage of being a compact unit that can be moved easily from one X-ray set to another. Its large active area and high spatial resolution also give it an edge in the large-unit-cell range.

The gain in signal-to-noise that detectors offer with respect to film can be used either for increased accuracy or for increased speed. The increased accuracy will be especially helpful for medium-to-large unit cells, where reliable heavy-atom differences have sometimes been difficult to measure and noisy difference data difficult to interpret. The increased speed offered by these new approaches to data collection is likely to have immediate impact in the analysis of variants, mutants, modified proteins, active-site complexes and the like. Since these aspects of an investigation follow the initial structure determination, they are usually not held up by the phase problem (for example, the search for heavy-atom derivatives) but rather by the process of intensity measurement itself. It should soon be much simpler to couple site-specific mutagenesis and X-ray crystallography, in order effectively to test hypotheses based on the examination of a new structure. □

1. Bragg, W.L. *Acta. crystallogr.* **A25**, 1 (1968).
2. Bragg, W.H. *Proc. Camb. phil. Soc.* **17**, 43 (1914).
3. Arndt, U.W. *Acta. crystallogr.* **B24**, 1355 (1968).
4. Arndt, U.W. *Nucl. Instrum. Meth.* **201**, 13 (1982).
5. Arndt, U.W. & Thomas, D.J. *Nucl. Instrum. Meth.* **201**, 21 (1982).
6. Xuong, N., Freer, S., Hamlin, R., Nielsen, C. & Vernon, W. *Acta. crystallogr.* **A34**, 289 (1978).
7. Hamlin, R. et al. *J. appl. Crystallogr.* **14**, 85 (1981).
8. Matthews, D.A. et al. *J. biol. Chem.* **253**, 6946 (1978).
9. Durbin, R.M., Burns, R., Harrison, S.C. & Wiley, D.C. *Proc. ACA Summer Meet.*, Snolomass, Colorado (1983).

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Linear scale for acid rain?

SIR — "Acid rain" is a scientific issue for which careful presentation of results is of considerable importance, especially as the issue has entered the domain public debate in the United Kingdom. It is therefore right to ask whether we always present the results of acidity measurements of rainfall and freshwaters in the most appropriate way. The answer, I believe, may be no.

The acidity of rainfall and freshwater bodies is commonly reported in terms of the pH . This is a long established scale and one that is particularly convenient for expressing concentrations of hydrogen (H^+) ions (strictly speaking H^+ activity) when they range over several orders of magnitude. Difficulties in its use can arise from a failure to recognize that, being logarithmic, the pH scale is not linear. Confusion on this count, particularly in nonscientific circles is easy to imagine. Even in scientific circles a drop in pH of say 0.5 units appears at times to be treated as being equivalent, whether it occurs from pH 6.0 or

last 400 years or so (Fig. 1a).

It is at this point, however, that caution needs to be exercised. Interpreting trends on the basis of the data as presented in Fig. 1a is not straightforward, due to the use of pH on the abscissa. Expressing the results in terms of H^+ ion concentration on a linear scale gives a significantly different impression of the trend over time in this lake (Fig. 1b).

To emphasize this point, the results in Fig 1a indicate that about half the total change in pH had taken place by around 1900. Furthermore, almost all of the change had taken place by 1930 with very little change after 1940. In other words the major pH change took place early in the first half of the present century. But in terms of H^+ ion concentration, half of the change had taken place by the later date of around 1920, while there was a further steep increase from around 1950 to the present (Fig. 1b).

The use of a linear representation of con-

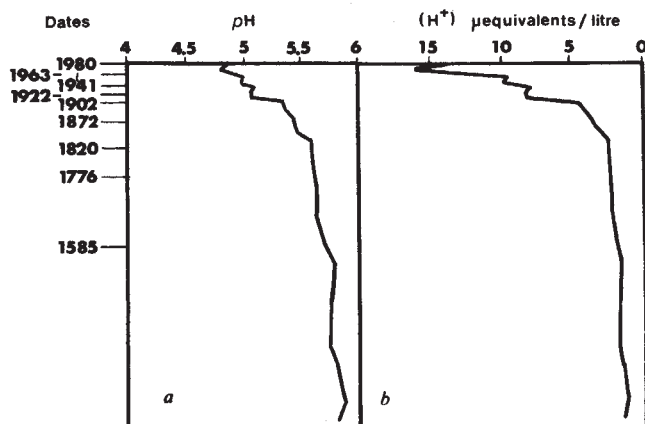


Fig. 1 Reconstruction of H^+ ion concentration history in Round Loch of Glenhead. a, Using pH scale (logarithmic); b, using linear concentration scale. (Based on ref. 1.)

from pH 5.0. In fact, the former case represents an increase in the H^+ ion concentration of some 2 microequivalents per litre, the latter of around 22 microequivalents per litre.

To give a more specific example, definition of trends over time in acidity levels has been commanding a great deal of attention. This is of some importance in establishing the relationship between emissions of sulphur dioxide and nitrogen oxides and subsequent acidification of the environment. Unfortunately, records of pH measurements of both rainfall and natural water bodies go back only several decades and even then there are problems over the continuity and quality of the data. Diatom stratigraphy in lake cores appears to provide one means of overcoming these problems, as demonstrated in the recent paper by Flower and Battarbee¹. By measuring the changes in the diatom population in a ^{210}Pb dated sediment core they were able to reconstruct the pH history of a lake in the south-west of Scotland over the

centrations provides the most appropriate means of representing the magnitude of any changes in concentration. (This is not to say though that effects are necessarily related linearly to concentration.) It is therefore encouraging to see a move to the use of a linear scale in reporting rainfall chemistry data in the United Kingdom². Indeed a widespread adoption of the linear concentration scale when reporting H^+ ion concentrations, especially in acidic rainfall and freshwaters, would appear to be overdue.

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1. Flower, R.J. & Battarbee, R.W. *Nature* **305**, 130-133 (1983).
2. Barrett, C.F. et al. *Acidity of Rainfall in the United Kingdom — A Preliminary Report* (Warren Spring Laboratory, Stevenage, 1982).

Alphabet and the Western mind

SIR — The unprecedented genius of Greece is unexplained. I conjecture that the modern study of the mind provides some tentative answers.

Before the Golden Age of Greece, the Greeks had developed their alphabet. This writing system is unique in possessing, by symbolizing vowels as well as consonants, a fully phonetic representation of language. This is achieved at the cost of limiting the representation of writing in the brain, as only the left cerebral hemisphere contains areas which are competent in the required phonetic analysis¹. This has led to the ubiquity of the association, in the alphabet-dominated modern world, between injuries to the left hemisphere and impairments of writing and reading skills. This, however, is not a necessary restriction with all writing systems. Non-phonetic writing systems can use reading strategies involving the right hemisphere. Indeed, neurological studies show that hemispheric representation of non-alphabetic writing systems differs markedly from that encountered with alphabetic ones. Hatta^{2,3} and others^{4,5} have shown that logographic characters are more efficiently recognized by the right hemisphere. Sasanuma⁶ has shown that *Kanji*, the non-phonetic writing system of Japanese, is vulnerable to different areas of brain injury from *Kana*, the phonetic system of Japanese writing.

There is evidence that reading retardation is associated with reduced involvement of the left hemisphere in reading⁷. In its absence such readers rely on the right hemisphere, which has been shown to have the ability to read simple English words independently of the left hemisphere⁸. In the absence of left-mediated phonetic analysis, the reading of frequent lexicals can occur through a non-phonetic strategy of logographically recognizing them from their shapes. It has been shown that retarded readers can learn to read Chinese logograms with their English translations more proficiently than their alphabetic equivalents⁹. Present studies of right hemispheric reading competence uses alphabetic lexicals which require left hemispheric phonetic decoding skills that disadvantage it rather than logographic ones which do not. I therefore suggest that the reading ability of right hemispheres for these and other non-Western writing systems will be found to have been underestimated. The right hemisphere, I conjecture, is fully competent, probably more so than the left hemisphere, to read, at the level of understanding which ancient societies used them, hieroglyphics, logograms and other non-alphabetic writing systems.

There are independent reasons for believing that pre-alphabetic writing systems were represented in the right hemi-

sphere. Such systems adopt a sinistral, that is, right to left, direction of writing and reading scan. Studies of eye scan direction have shown that sinistral scan direction favours a perception of lexicals to the left of the visual fixation point⁹. Such displacement of lexical perception into the left visual field would be expected if lexicals were preferably processed in the right hemisphere. Likewise as dextral scanning produces a perception of lexicals to the right of the fixation point, the dextrality of the Greek alphabet would follow from the adoption of a left hemispheric perception of lexicals through the right visual field.

Why should the development of a left rather than a right or bilateral representation of writing, as a consequence of the alphabet, have led to the Greek intellectual revolution? The answer probably lies in the fact that "competences" within the brain compete for expression. Analogously to Mendelian genes, the competences of the mind can have a dominant or recessive relationship to each other. Levy and Trevarthen⁹ have shown that when two hemispheres each possess a competence able to answer a question, only one will do so. Though often the dominant competence will be superior in correctly replying compared with the recessive competence it dominates, frequently the recessive competence, as found by unilaterally testing it, will have a superior ability to answer to the question correctly than the competence which dominates it.

I conjecture that the alphabet, by producing a unilateral representation of lexicals in the left hemisphere, freed recessive competences in the left hemisphere which underlie rational, analytical and logical thought. These competences were previously overshadowed, inhibited and restricted in their development by a right hemispheric set of competences deriving from its representation for lexicals.

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1. Ojemann, G.A. *Behav. Brain Sci.* 6, 189-230 (1983).
2. Hatta, T. *Neuropsychologia* 14, 685-688 (1977).
3. Hatta, T. *Neuropsychologia* 19, 87-94 (1981).
4. Tsao, Y.-C. *et al. Brain Lang.* 8, 367-371 (1979).
5. Sasanuma, S. *et al. Neuropsychologia* 15, 547-554 (1977).
6. Sasanuma, S. & Fujimura, O. *Cortex* 7, 1-18 (1971).
7. Marcel, T. *et al. Neuropsychologia* 12, 131-139 (1974).
8. Levy, J. & Trevarthen, C. *Brain* 100, 105-118 (1977).
9. Rozin, P. *et al. Science* 171, 1264-1267 (1971).
10. Pollatky, A. *et al. Brain Lang.* 14, 174-180 (1981).
11. Levy, J. & Trevarthen, C. *J. exp. Psychol. hum. Percept. Perform.* 2, 299-312 (1976).

Alignment of base sequences

SIR — In a recent paper Richards *et al.* show¹, in their Fig. 4, an alignment in which they claim that gaps "were inserted to maximize the number of base matches". The authors are to be commended on the clarity of phrase "number of base matches" when so many would have preferred the ambiguous term "homology". They have, nevertheless,

still managed to mislead the careless reader as the following segment of their alignment shows:

```
-----CCTTCAGAAATACAGAAATAGGGACATAGAGA
ATCCGACCCAGCCCCCTGGACCTGTAT-----
```

(Underlining shows the base matches.) An alternative alignment might be as follows:

```
CCTTCAGAAATACAGAAATAGGGACATAGAGA
ATCCCA-----CCCAGCCCCCTGGACCTGTAT
```

This alignment reduces the number of gaps in this region by 50 per cent, reduces the number of gapped residues by 80 per cent, and increases the number of base matches by 133 per cent. More modest improvement around two of their other gaps is also possible. If I can increase the number of base matches by removing gaps, then clearly the authors' insertion of gaps did not maximize that number. One should seldom claim that one has maximized anything in the absence of a rigorous algorithm to accomplish that task². It is sufficient (and frequently correct) to assert that gaps have been introduced to increase the number of base matches.

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1. Richards, J.E., Gilliam, A.C., Shen, A., Tucker, P.W. & Blattner, F.R. *Nature* 306, 483 (1983).
2. Smith *et al. J. molec. Evol.* 18, 38 (1981).

Fissure animals of Bristol and South Wales

SIR — Michael Benton has given a fair account of the published material on the Upper Triassic and Lower Jurassic fissures of Bristol and South Wales¹. However, in reviewing my work on the fissures, he missed some important aspects.

He states that "we must now view the fissure animals as representing a sample of a lowland fauna rather than a peculiar upland fauna". In particular, the tetrapods should be viewed as representing an insular lowland fauna and not the usual continental lowland fauna. The poorly dated mammal-bearing fissures of South Wales are generally described as being on islands^{2,3} of (probably) Lower Liassic Age, whereas the specifically dated⁴ Tytherington fissure fauna can be viewed as being derived from a small low-lying Rhaetian island habitat⁵. Indeed, the incidence of these tetrapod faunas, which are dominated by species with a small body size, is remarkably similar to Quaternary herpetofaunas found in solution fissures⁶ or banana-holes⁷ of modern-day islands.

The importance of this concept, that the Bristol and South Wales fissure animals are derived from insular Triassic/Liassic faunas, cannot be underestimated. Because of the small land areas on which they live each reptile species would have had a relatively low overall population size and thus would be subject to rapid rates of genetic drift and a high probability of ex-

inction. Insular faunas are impoverished and show evidence of disharmony⁸. Therefore the biota could be expected to be unusual in respect to other preserved faunas of the same age, and any given fissure reptile species may show aberrant features compared to its contemporaneous unpreserved relatives. In this respect, rather than being closely related to *Anchisaurus* as generally proposed⁹, *Thecodontosaurus* is more likely to be a dwarf plateosaurid (either *Plateosaurus* or *Lufengosaurus*). Full details are in preparation but, for example, it is the case that the basioccipital and basisphenoid of *Thecodontosaurus antiquus* discovered at Durdham Down is much more similar to that of *Plateosaurus* than that of *Anchisaurus*.

The high turnover rates expected on these small limestone islands lying near the Welsh and European landmass would also provide one alternative interpretation of the differences between the limestone faunas of the Slickstones fissures described by Fraser and Walkden¹⁰.

The remarkable preservation of the many tiny reptile bones can easily be explained by the short transport distances from death to burial expected in small islands. Indeed, deposition of the bones may be at least partly below sea level (see ref. 5).

Finally, reptile remains were described from Durdham Down fissure¹¹, and lepidosaur vertebrae were found in Holwell¹²; both these reports predate the description of *Clevosaurus* in 1939, that Benton refers to as the earliest description of reptile remains from these fissure deposits.

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1. Benton, M. *Nature* 307, 111-112 (1984).
2. Robinson, P.L. *Palaeontology* 14, 131 (1971).
3. Evans, S.E. *Zool. J. Linn. Soc.* 70, 203 (1980).
4. Marshall, J.E.A. & Whiteside, D.I. *Nature* 287, 627 (1980).
5. Whiteside, D.I. & Robinson, D. *Palaeogeogr., Palaeoclimat., Palaeoecol.* 41, 81 (1983).
6. Taylor, J.D., Braithwaite, C.J.R., Peake, J.F. & Arnold, E.N. *Phil. Trans. R. Soc. B* 286, 47 (1979).
7. Etheridge, R. *Q. J. Fla. Acad. Sci.* 28, 349 (1966).
8. Williamson, M. *Island Populations* (Oxford University Press, 1981).
9. Galton, P.M. & Cluver, M.A. *Ann. S. Afr. Mus.* 69, 121 (1976).
10. Fraser, N.C. & Walkden, G.M. *Palaeogeogr., Palaeoclimat., Palaeoecol.* 41, 81 (1983).
11. Riley, H. & Stutchbury, S. *Proc. geol. Soc.* 2, 397 (1836).
12. Owen, R. *Q. J. Geol. Soc. Lond.* 16, 492 (1860).

Seminal lymphocytes, plasma and AIDS — Erratum

IN the piece of "Scientific Correspondence" by Keith James in the issue of 10 May (*Nature* 309, 117; 1984) the reference list was omitted. It should read:

1. Prakash, C. & Lang R.S. *Mt. Sinai J. Med.* 47, 491 (1980).
2. Anderson, D.J. & Tarter, H. *J. Immun.* 128, 535 (1982).
3. James, K., Bradbury, A. W., Hargrave, T.B., Cullen, R.T. & Donaldson, K. W. *AIDS Res.* 1, 45 (1983).
4. Talal, N. *Immun. Today* 4, 180 (1983).
5. Roder, J.C. & Haliotis, T. *Immun. Today* 1, 96 (1980).
6. James, K. & Ritchie, A.W.S. *Immun. Today* (in the press).
7. Peterson, B.H., Lammel, C.J., Stites, D.P. & Brooks, G.F. *J. Lab. clin. Med.* 96, 682 (1980).
8. Wilkin, S., Richards, J.M., Bongiovanni, A.M. & Zelickovsky, G. *Am. J. Reprod. Immun.* 3, 23 (1983).

Stoichiometry and the structure and stability of inorganic solids

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Stoichiometry (cation/anion ratio) is an important factor in determining the structure and stability of many inorganic crystals. It directly affects the coordination numbers of atoms, the relative stability of oxidation states and the tendency to form compounds with bonds between like atoms. Cation-rich binary compounds, especially oxides and nitrides, are either relatively unstable (have reduced bond energies) or do not exist. In such cases peroxides and azides are preferentially formed; these have normal bond energies.

WE are concerned here, in a very general way, with the role of stoichiometry in determining the structure and stability of simple inorganic solids. Although elementary, its importance in chemistry has generally been underestimated (but see ref. 1). We find that several observations, otherwise not readily explicable, are susceptible to a simple rationalization in terms of the constraints imposed by stoichiometry (which are ultimately those of valence). The emphasis is mainly, but not exclusively, on binary compounds of main group metals with nitrogen, oxygen and fluorine.

We first distinguish between normal compounds and polycompounds. The former are those in which there are bonds only between unlike atoms (as in SiO_2). In the latter² there are also identifiable bonds between like atoms (as in azides and peroxides). These terms provide at best a rough classification; in general there is no rigorous criterion for deciding whether any two atoms in a molecule or a crystal are, or are not, bonded together. It is well known^{3,4}, for example, that bond-order criteria based on interatomic distances (d) often lead to results contrary to intuition. (For example, in Mg metal, $d(\text{Mg-Mg}) = 3.21 \text{ \AA}$, whereas in MgO , $d(\text{Mg-Mg}) = 2.98 \text{ \AA}$.) Equally, it is now recognized⁵ that the presence of a build-up of electron density between atoms does not automatically indicate the presence of covalent bonding.

In the normal compounds of the main-group elements, the oxidation states are mainly determined by the position of the element in the Periodic Table. Accordingly, there is usually just one normal binary composition for a given pair of elements (such as, Cs_2O), although the compound may well be polymorphic. (There are obvious exceptions involving mainly pairs of the more-electronegative elements such as the sulphur or nitrogen oxides.) On the other hand, the same pair of elements may well form a number of polycompounds such as Cs_3O , Cs_2O_3 , Cs_{11}O_3 and Cs_7O . Note that $\text{Cs}_2\text{O}_3 [= \text{Cs}_4(\text{O}_2)_3]$ has both polycations and polyanions.

Pairs of elements which form stable normal compounds, very often do not form polycompounds [for example, AlN but not $\text{Al}(\text{N}_3)_3$] and conversely, those which form stable polycompounds often do not form stable normal compounds (for example, NaN_3 but not Na_3N). In what follows we generally discuss stability with reference to dissociation to free atoms (bond energies); although by this test Na_3N may well be stable, however, we shall argue that in this instance the bond energy is unusually low.

Our approach is largely phenomenological. In particular we will not discuss whether compounds are 'ionic' or 'covalent', although for convenience we shall use the terms *anion* and *cation*

when the context leaves no room for ambiguity. We shall, however, offer an interpretation of our observations on the properties of metal-rich compounds (those with cation/anion stoichiometric ratios significantly greater than one) in which we identify a destabilizing influence of cation-cation interactions. Here a 'size' effect clearly emerges.

Bond energies and strengths

The term bond strength is used as defined by Zachariasen⁶, so that for bond strengths v_{ij} between atom i and neighbours j

$$\sum_j v_{ij} = z_i \quad (1)$$

where z_i is the valence of i . For a symmetrical coordination polyhedron v is the Pauling⁷ electrostatic valence. In the absence of large interactions between next-nearest neighbours, v_{ij} is, to a good approximation, a unique function of the bond length r_{ij} .

Elsewhere⁸ we have used the concept of equivalent bond enthalpy (the bond dissociation energy per unit bond strength) and demonstrated that (with exceptions of special interest below) it is approximately constant for bonds to a given metal in oxides. The enormous number of natural chemical compounds shows that equivalent bond enthalpies must be approximately constant for any pair of elements.

In rare instances, for example CO_2 , high order ($v > 1$) bonds are favoured. More commonly, the situation is one in which structures with many weaker bonds are favoured to an alternative with fewer strong bonds: an example is SiO_2 . Thus, in general, large coordination numbers are preferred, and this gives rise, for most combinations of elements, to solid structures. Fragmentation of structures into molecules, with low coordination numbers, is the exception, and occurs only with relatively few combinations of elements. We are mainly concerned, therefore, with the factors that provide upper limits to coordination numbers.

Coordination numbers in crystals

In most crystals, coordination numbers are determined by three principal factors. The first two involve a balance between the tendency to form as many bonds as possible, and the interactions (normally, but not necessarily, repulsions) between the next near-nearest-neighbour atoms. Note that when considering such interactions, the 'size' of an atom is its non-bonded size⁹, which has no relationship to conventional ionic radii. Indeed, the failure of the ionic radius ratio rules for coordination numbers has long been recognized¹⁰, but still not generally accepted. This neglect has led to the erroneous point of view that (to take a specific example) cation coordination numbers in oxides are

solely determined by oxygen–oxygen repulsions and not by cation–cation repulsions.

An important point is that cation and anion coordination numbers are not independent: by definition, each bond has a cation at one end and an anion at the other, so that the ratio of the average coordination number of the anions to that of the cations is equal to the stoichiometric cation/anion ratio¹¹. Thus is SiO_2 , ${}^{\text{iv}}\text{Si}$ implies—at least on average— ${}^{\text{ii}}\text{O}$, and we may ask whether the coordination number of Si in SiO_2 is limited to four (at ordinary pressure) because no more than four oxygen atoms can fit around a silicon atom, or because no more than two silicon atoms can fit around an oxygen atom. We prefer the second point of view⁹.

In order that the shortest interatomic distances in normal crystals be between cations and anions, coordination numbers must clearly be less than, or equal to, 12. In binary compounds the limiting value is only reached in exceptional structures such as those of Cr_3Si and WAl_{12} (hardly normal compounds in the present context). In practice this third constraint limits coordination numbers in normal compounds of nitrogen, oxygen and fluorine so that values in excess of nine are very rare. It follows that when the cation/anion ratio is very different from unity, the majority component will have a rather low coordination number (thus four for Rb in Rb_2O and two and three for Li in Li_3N). This important influence of stoichiometry in influencing coordination numbers appears largely to have gone unrecognized although N. V. Belov (quoted by Lebedev¹⁰) apparently realized, but did not explain, the phenomenon.

Examples of unusual coordination numbers are to be found among the many cation-rich ternary oxide structures determined by Hoppe and coworkers¹². Thus transition metal cations in planar 3-fold coordination are found in Na_4MO_3 ($M = \text{Zn}, \text{Fe}, \text{Co}$ and Ni) and in $\text{Na}_{10}\text{Co}_4\text{O}_9$, and $\text{Ni}(\text{II})$ is in linear 2-fold coordination in K_2NiO_2 . We emphasize that these low coordination numbers for the cations are due in large part to the high stoichiometric ratio of cations to anions in the compounds.

We have mentioned that in SiO_2 , the coordination number of Si was largely determined by the difficulty of fitting three Si atoms around an oxygen atom. This is neatly illustrated by the structure of SiP_2O_7 , in which the increased anion/cation ratio allows silicon to be six coordinated by oxygen while keeping the oxygen coordination at two (that is ${}^{\text{vi}}\text{Si}{}^{\text{iv}}\text{P}_2{}^{\text{ii}}\text{O}_7$). There are several other silicon phosphates in which the coordination number of silicon is similarly controlled by stoichiometry¹³.

Irregular coordination figures

The occurrence of irregular coordination figures in structures can also be a simple and inevitable consequence of stoichiometry. We illustrate this point by stoichiometry M_2X_3 . If M is to have coordination number four, X atoms must exist in at least two different coordinations (the average coordination number of X being $8/3$). Thus ${}^{\text{iv}}\text{B}_2{}^{\text{ii}}\text{O}{}^{\text{iii}}\text{O}_2$ for the structure of high-pressure B_2O_3 . In this compound the sum rule, equation (1), requires that the strengths of the bonds to the two-coordinated oxygen are $v = 1$ and for the three-coordinated oxygen, $v = 2/3$. As bond strengths and bond lengths are strongly correlated¹⁴ these two bond strengths correspond to different bond lengths—in this example 1.38 Å and 1.51 Å respectively. The coordination figure about boron ($\text{B}{}^{\text{iv}}\text{O}{}^{\text{iii}}\text{O}_3$) is correspondingly an irregular tetrahedron. The irregular coordination figure about La in A-type La_2O_3 (in which La is in 7-fold coordination) is similarly explained.

In such structures, the Pauling⁷ electrostatic valence sum rule is not obeyed, and according to Pauling's hypothesis they are destabilized. Certainly there are no stable oxides of stoichiometry M_2O_3 in which all the M atoms are in four-coordination despite the fact that atoms such as B, Al, Ga, Fe(III) are frequently in four coordination in ternary oxides and such like. We caution against too much generalization; for some metals (notably the early transition elements) distorted coordination polyhedra are in fact more stable than regular ones (as illustrated by the structures of MoO_3 and WO_3). Nevertheless,

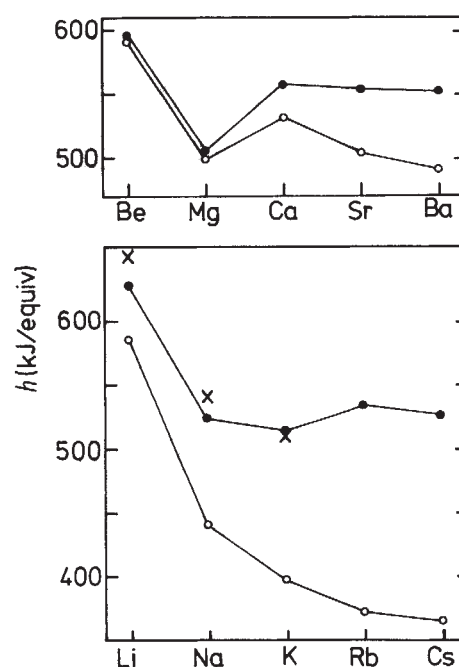


Fig. 1 The equivalent bond enthalpy, h , in alkali and alkaline earth oxides. ●, Ternary and similar oxides⁸; ○, normal binary oxides; ×, peroxides.

the instability of irregular coordination figures of the type just discussed is the reason for some departures from the general rule that cation coordination decreases with increasing cation/anion ratio. An obvious example is ${}^{\text{iv}}\text{AlPO}_4$ and ${}^{\text{vi}}\text{Al}_2\text{O}_3$; another is the structure¹⁵ of Na_3NO_4 with ${}^{\text{iv}}\text{N}$ (nitrogen is considered a cation in this context) in contrast to ${}^{\text{iii}}\text{N}$ in NaNO_3 .

Bond energies

An earlier study⁸ of heats of atomization of oxides showed that there is a constant contribution, per unit of bond strength, for each M–O bond, to the total heat of atomization of ternary and similar oxides. With the exception of the alkali metals and, to a lesser extent, the heavier alkaline earth metals, the contribution from each element was equal to the equivalent heat of atomization of its binary oxide.

The data of that study (for Li, Na and K) have now been augmented with the few available data for ternary oxides of Rb and Cs (ref. 16). These have been used to calculate the equivalent bond enthalpies both in binary and in ternary and such like oxides that are shown in Fig. 1. Note that these quantities differ by an amount that increases with increasing size (atomic number) of the metal atom. The most obvious way that the binary oxides differ from the rest is in stoichiometry; the latter all had cation/anion ratios less than one, in the former it is two. We suggest that the higher proportion of metal atoms in the binary oxides result in significant cation–cation interactions which destabilize the crystal to an extent that increases with cation size. This view is strongly supported by the observation¹⁷ that bond lengths in alkali metal oxides are anomalously lengthened (compared with those in ternary compounds) in a parallel manner.

Figure 1 also shows the M–O equivalent bond enthalpies in the peroxides M_2O_2 . These were calculated from the heats of atomization of the solid peroxides by subtracting the O–O bond energy, the latter coming in turn from the heats of atomization of H_2O and H_2O_2 (ref. 18). It may be seen that they are normal in the sense that they lie very close to the points found for the ternary oxides, showing clearly that the increasing tendency to peroxide formation with increasing atomic number is due, not to enhanced stability of the peroxide, but rather to decreased stability of the normal binary oxides.

For the nitrides and azides fewer relevant data are available. However, Wattenberg's¹⁹ work shows that if it exists at all, Na_3N

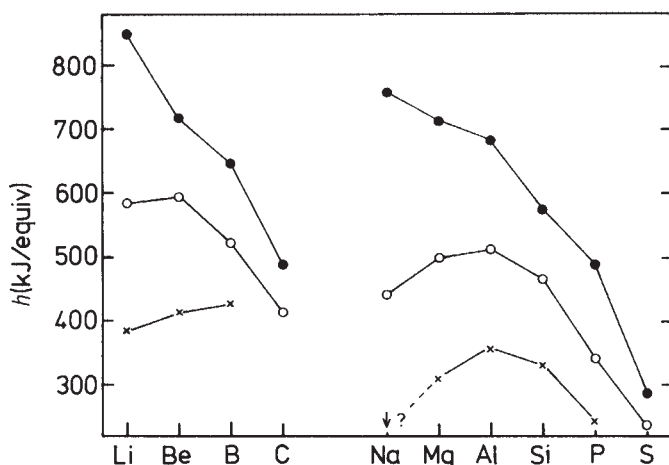


Fig. 2 Equivalent bond enthalpies (heats of atomization per equivalent) for binary solids. ●, Fluorides; ○, oxides; ×, nitrides. The arrow represents the upper limit for Na_3N derived in the text.

is metastable with respect to $\text{Na}(\text{metal})$ and N_2 . Thus for the reaction $6\text{Na} + \text{N}_2 = 2\text{Na}_3\text{N}$, $\Delta G_{273}^0 > 0$ from which we estimate $\Delta H_{273}^0 > -57 \text{ kJ mol}^{-1}$, and hence an upper limit for the equivalent bond enthalpy in the binary compound $h_0(\text{Na-N}) < 260 \text{ kJ mol}^{-1}$. A value for the equivalent bond enthalpy $h(\text{Na-N})$ in Na_3N may be obtained in the same manner as for the peroxides (that is using data for NaN_3 , HN_3 and NH_3) giving $h = 554 \text{ kJ mol}^{-1}$. Although rough estimates, the difference $h - h_0$ is striking and demonstrates that sodium nitride, like sodium oxide, has an anomalously low bond energy.

Figure 2 shows equivalent bond energies for normal binary compounds with N, O and F. The fluorides show the expected trend of bond energy increasing with increasing electronegativity difference. However, bond energies to N, and to a lesser extent, to O are clearly anomalous in the case of Na and, to a lesser extent Mg. (In contrast, the equivalent bond energies in ternary, and similar oxides⁸ show the expected trend similar to that of the fluorides in Fig. 2).

Nonexistent compounds

We have called attention to the reduced stability (and consequent lengthened bonds) in certain metal-rich normal compounds. The effect was larger for heavier metal atoms and larger metal/non-metal ratio. It might then be expected that certain compositions are not realized as stable phases. This indeed occurs. It is also found that when the normal compounds do not exist, stable polycompounds are the rule. We are particularly concerned with providing a simple rationalization for the non-existence of normal binary compounds which might be expected to be stable on the basis of a large difference of electronegativity between the two components.

It is at first sight surprising that so few normal carbides of the main group elements are known. These seem to be restricted to SiC , Al_4C_3 , Be_2C and, possibly²⁰, Li_4C . The other alkali metals readily form graphite intercalation compounds (a form of polycompound) and acetylides but not solid M_4C . The heavier alkaline earth metals form only acetylides. It seems clear that these observations may be simply and directly explained as a consequence of the large number of metal-metal interactions that would be imposed by the stoichiometry of the missing compounds. The non-existence of other compounds such as GeC may be ascribed to the same cause. Here we are noncommittal about the nature of metal-metal 'interactions'; molecular orbital calculations²¹ on molecules such as Li_4C and Li_3N show signs of a bonding overlap population between the lithium atoms and indeed hypervalent molecules such as Li_6C are also predicted²² to be stable (and here there are clear indications of Li-Li bonding).

In contrast to the carbides, all the alkaline earth metals form normal silicides (increasing bond lengths act to mitigate

metal-metal interactions). Silicon and aluminium do not react, but the difference in electronegativity is becoming rather small.

We have already remarked on the relative instability of Mg_3N_2 and by inference of the other alkaline earth nitrides, and of Li_3N . It is clear¹⁸ that the other alkali metals do not form normal nitrides, but they all form stable azides. The size effect is also apparent here in that the rest of the normal alkali metal pnictides are well characterized¹.

The complementary instability of at least some polycompounds can again be ascribed to the constraints of stoichiometry²; $\text{Al}(\text{N}_3)_3$ for example, would be very crowded indeed—but now with anions rather than cations. The problem of the stability of peroxides and azides is closely analogous to that of the stability of oxysalts—for example, the only stable anhydrous perchlorate are those of the alkali metals and the alkaline earths other than beryllium²³. Wells¹ has discussed the stability of such salts from a similar point of view to ours.

Oxidation states

We have presented quantitative evidence that cation-rich oxides have abnormally low stabilities. In the case of ternary transition metal oxides, it is clear that the cation/anion ratio could be decreased (and the stability increased) by increasing the oxidation state of the cation. Alternatively, a high oxidation state of a transition metal could be stabilized by forming a ternary oxide with a low valence metal, and, indeed, high oxidation states of transition metals are readily obtained in ternary alkali-metal oxides. Thus although the highest stable oxidation states in the binary oxides are Mn(IV) and Ni(II), KMnO_4 [with Mn(VII)] and Na_5NiO_4 (ref. 24) [with Ni(III)] are known.

Conversely, higher oxidation states are destabilized by a large anion/cation ratio. Despite the (frequently correct) assertion that fluorine tends to develop the highest valence of a cation [thus Ni(IV) in ternary fluorides²⁴], this is not the case for transition metal cations having the potential of high valence. For example, CrO_3 is known but not CrF_6 (ref. 25). The oxidizing power of fluorine is allowed full rein when combined in ternary fluorides with alkali and alkaline earth metals (especially the heavier ones). Then we find, for example, Cs_2MF_6 with $\text{M} = \text{Cu}(\text{IV})$ or $\text{Ag}(\text{IV})$ [contrast M(II) as the highest oxidation state in the binaries] and CsAuF_6 with Au(V) (ref. 24).

Conclusions

We have shown how the constraints of stoichiometry affect both the structure and stability of simple inorganic solids. Although simple and obvious, our ideas provide a unifying understanding of a range of phenomena that have not previously been explained, except perhaps in isolated instances on an *ad hoc* basis. We have adduced only a few more-striking examples to illustrate our thesis. But we have not omitted any counter-examples known to us.

There have, of course, been numerous previous attempts at developing useful generalizations concerning the relative stability of inorganic solids. One of the first and most successful was that of Ramberg²⁶, who compared the relative stabilities of pairs of salts, and deduced that the low-energy assemblages were those in which the most highly charged cations were combined with the most highly charged anions (that is, $\text{Mg}_3\text{N}_2 + \text{LiCl}$ rather than $\text{Li}_3\text{N} + \text{MgCl}_2$)—the interpretation of such rules in terms of the ideas we have set out here should be clear.

It should also be clear that we provide a rationalization for the observation that the heats of reaction between acidic oxides and basic oxides is large, for example,



whereas that between amphoteric oxides is small, for example,



However, note that many of the observations that we have made are not in accord with the generalizations of the theory of hard

and soft acids and bases (HSAB)²⁷ which has several features in common with Ramberg's treatment. Thus according to the HSAB approach, hard acids such as Na should form more stable compounds with hard bases such as N than with weak bases such as As. Our discussion shows that the converse is true. Another prediction²⁸ of the HSAB theory is that to stabilize an

element in a high oxidation state it should be surrounded by hard bases such as O²⁻. We have seen that in solid compounds, the addition of hard acids such as Na⁺ is, in fact, what is required.

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1. Wells, A. F. *Structural Inorganic Chemistry* 4th edn (Clarendon, Oxford, 1975).
2. Hulliger, F. in *Structure and Bonding in Crystals* Vol. 2 (eds O'Keeffe, M. & Navrotsky, A.) 297-352 (Academic, New York, 1981).
3. Corbett, J. D. *J. Solid St. Chem.* **37**, 335-351 (1981).
4. Simon, A. *Angew. Chem.* **22**, 95-113 (1983).
5. Dunitz, J. D. & Seiler, P. *J. Am. chem. Soc.* **105**, 7056-7058 (1983).
6. Zachariasen, W. H. *Acta crystallogr.* **16**, 385-389 (1963).
7. Pauling, L. *The Nature of the Chemical Bond* (Cornell, New York, 1960).
8. O'Keeffe, M. & Stuart, J. A. *Inorg. Chem.* **22**, 177-179 (1983).
9. O'Keeffe, M. & Hyde, B. G. in *Structure and Bonding in Crystals* (eds O'Keeffe, M. & Navrotsky, A.) 227-254 (Academic, New York, 1981).
10. Lebedev, V. I. *Dokl. Akad. Nauk, SSSR* **188**, 666-669 (1969).
11. O'Keeffe, M. & Hyde, B. G. *J. Solid St. Chem.* **44**, 24-31 (1982).
12. Hoppe, R. *Angew. Chem.* **19**, 110-125 (1981).
13. Mayer, H. *Monatsh. Chem.* **105**, 46-54 (1974).
14. Brown, I. D. in *Structure and Bonding in Crystals* Vol. 2 (eds O'Keeffe, M. & Navrotsky, A.) 1-30 (Academic, New York, 1981).
15. Jansen, M. *Angew. Chem.* **18**, 698-699 (1979).
16. Kubaschewski, O., Evans, E. L. & Alcock, C. B. *Metallurgical Thermochemistry* 4th edn (Pergamon, Oxford, 1967).
17. McGuire, N. & O'Keeffe, M. *J. Solid St. Chem.* (in the press).
18. Stull, D. R. & Prophet, H. (directors) *JANAF Thermochemical Tables* (National Bureau of Standards, Washington, 1971).
19. Wattenberg, H. *Ber. dt. chem. Ges.* **63**, 1667-1672 (1930).
20. Chung, C. & Lagow, R. J. *JCS chem. Commun.*, 1079-1080 (1972).
21. O'Keeffe, M. & MacMillan, P. F. (in preparation).
22. Schleyer, P. v. R., Würthwein, E.-U., Kaufmann, E., Clark, T. & Pople, J. A. *J. Am. chem. Soc.* **105**, 5930-5932 (1983).
23. Solymosi, F. *Structure and Stability of Salts of Halogen Oxyacids in the Solid Phase* (Wiley, New York, 1977).
24. Hoppe, R. *Angew. Chem.* **20**, 63-87 (1981).
25. Dasent, W. E. *Nonexistent Compounds* (Dekker, New York, 1965).
26. Ramberg, H. *The Origin of Metamorphic and Metasomatic Rocks* (University of Chicago Press, 1952).
27. Pearson, R. G. *Science* **151**, 172-177 (1966).
28. Pearson, R. G. *Chem. Br.* **3**, 103-107 (1967).

ARTICLES

Optical counterparts of unidentified IRAS point sources: infrared luminous galaxies

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Deep charge-coupled device imaging has been used to identify a number of IRAS (Infrared Astronomical Satellite) blank field sources with distant galaxies, and indicates that most high-latitude unidentified IRAS point sources have an extragalactic origin. The present sample of galaxies seems to include some with the largest known ratios of IR-to-optical luminosity, with values of L(IR)/L(B) ranging upwards of 200. A redshift obtained for one source, 0422+009, indicates that this object has ~35 times the absolute IR luminosity of the starburst galaxy M82.

HOUCK *ET AL.*¹ have recently reported several point-like 60- μ m sources with no obvious visible counterparts. Possible explanations for the nature of these sources include high redshift galaxies, heavily obscured young stars, evolved stars with circumstellar dust shells, nearby 'Jupiter-like' bodies, or some fundamentally new type of object. To help establish which of the possibilities is correct, we have obtained deep, near-red images centred on the Houck *et al.* source positions.

CCD imaging

Houck *et al.* present nine unidentified sources: six near RA 4 h, and three near RA 17 h. On the night of 9-10 December 1983, the prime focus charge-coupled device (CCD) camera of the 4-m telescope at Cerro Tololo was used to observe five of the RA 4 h fields. Four-minute *I* band exposures were obtained for each field, and for three of the sources, 5-min *R* band exposures were also secured.

Images of the observed fields are presented in Fig. 1. Following standard procedures², the CCD data have been trimmed, debiased, flattened to ~0.3% across the frame by exposures on both dome and smoothed blank sky, and corrected for fringes arising from night sky emission. Low-level (~1%) fringing remains in the data. Also shown in Fig. 1 are the IRAS error ellipses from Houck *et al.*¹, together with several galaxies that we have found lying within or near the ellipses. An examination of the radial brightness profile easily distinguishes non-stellar

from stellar objects in our data. We first discuss each of the six RA 4 h sources.

0358+223. No CCD data were obtained for this field. However, a bright, stellar-like object is visible on the Palomar Observatory Sky Survey (POSS) at the virtual centre of the IRAS error ellipse¹. Careful examination of POSS plates stored at Kitt Peak shows that the image of this object is 'soft', indicative of a distant galaxy which we identify with the IR source. The POSS prints examined by Houck *et al.*¹ are presumably inadequate for such fine discrimination.

0404+101. Figure 1 shows a faint galaxy near the centre of the IRAS error ellipse which we identify as the IR source. This object is visible just at the red plate limit of the POSS.

0412+085. We do not identify this source with any obvious optical counterpart. A faint galaxy does fall on the edge of the error ellipse, but we are hesitant to claim correspondence with it. First, because the IR-to-optical luminosity ratio would be uncomfortably large, even in comparison with the other great values derived later here; and more important, the probability of chance superposition for galaxies this faint becomes significant³. Further, re-examination of the IRAS data indicates that the source is probably extended, and therefore likely to be a hotspot on the IR cirrus (C. A. Beichmann, personal communication) present in this region (see ref. 4). The source has, in fact, the lowest signal-to-noise ratio of those reported by Houck *et al.*¹. If it eventually does prove point-like, correspondence with

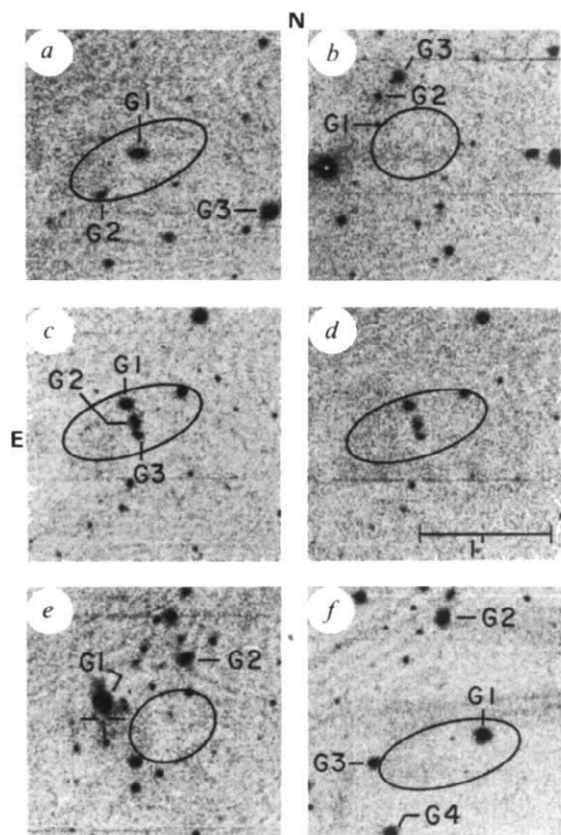


Fig. 1 CCD images of: a, 0404+101 (R band); b, 0412+085 (I band); c, 0413+122 (R band); d, 0413+122 (I band); e, 0422+009 (R band); and f, 0425-012 (I band). IRAS error ellipses from Houck *et al.*¹ are also shown. The alternative IRAS position for 0422+009 is marked in e. Galaxies are numbered as in Table 1. The orientation and scale for all images is identical.

galaxy number 3 may remain a possibility, although this object does fall a long way out of the present error ellipse.

0413+122. A galaxy triplet is located in the IRAS error ellipse. We suspect that galaxy number 2, near the centre of the ellipse, is the IR source. This object looks obviously disturbed, and the slightly distorted shape of galaxy number 3 is suggestive of tidal interaction between the two. The asymmetrical appearance of galaxy number 2 may be partially due to what looks like either a dust lane running along the north-east side of the object, or to two galaxies being viewed in close superposition. The triplet is again just visible at the POSS red-plate limit.

0422+009. Figure 1e shows that no obvious counterpart is present in the original IRAS error ellipse. However, an alternative source position obtained from later scans of the area (C. A. Beichmann, personal communication) places the IR source nearly coincident with a galaxy that appears to be the brightest member of a small cluster. Given the odd morphology and rather 'dusty' appearance of this object, we conclude that it is in fact the IR source (although there appears to be no *a priori* reason for preferring either of the two source positions marked in Fig. 1e).

0425-012. The radial profile of the stellar-like object in the error ellipse (see also ref. 1) establishes it as a galaxy, which we identify with the IR source. The image on the POSS red plate is also soft, although less obviously so than for 0358+223. (We have also examined the POSS plates at the positions of the three RA 17h sources listed by Houck *et al.*¹, and find that galaxies appear to be present in at least two of the error boxes.)

We now need to assess the probability of chance background contamination. For this purpose we use the r_F counts of Shanks *et al.*³; over the colour range of interest, $r_F \approx R$ magnitudes on

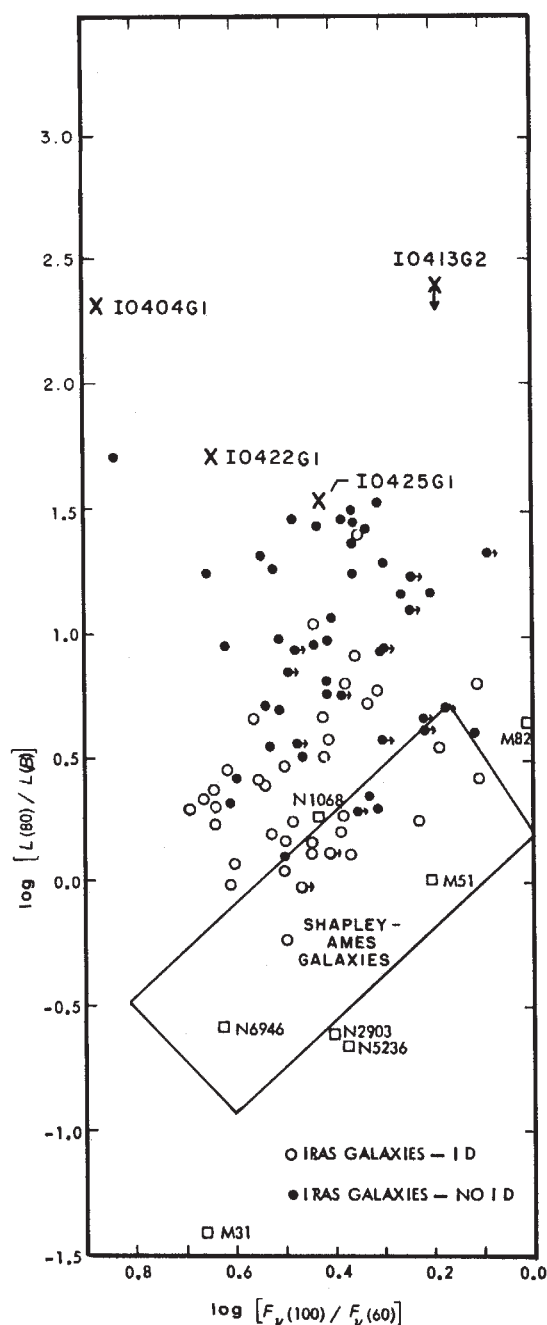


Fig. 2 The ratio of luminosity at 80 μm to that in the blue is plotted against the flux density ratio at 100-60 μm . $\times$, Galaxies studied here; $\circ$, catalogued and $\bullet$, uncatalogued galaxies in the IRAS mini-survey². The box encompasses the location of most Shapley-Ames galaxies¹⁰. Some selected nearby bright galaxies are also individually labelled.

the Kron-Cousins system, which we use for the photometry. Of the five IRAS sources we have identified, three have an estimated $R \leq 17.5$ mag, while two have an estimated $R \sim 19$ mag. For the typical error ellipse area of ~ 0.5 arc min², we find a probability of ~ 0.03 for random coincidence if $r_F \leq 17.5$ mag, which becomes ~ 0.16 for $r_F \leq 19$ mag. On an individual basis, then, the probability of chance background contamination is low; but, on the other hand, we can expect to find one interloper having $r_F \leq 19$ mag given six total surveyed fields. Hence, one of our suggested identifications may be spurious. This question can be further considered when more accurate IRAS source positions become available, but we believe that at present the source identifications given above are in most (and possibly all) cases correct.

We conclude that distant galaxies probably dominate the sample of unidentified point sources presented by Houck *et al.*¹, rather than galactic objects or some previously undiscovered type of source. Indeed, we have located galaxian counterparts for all five of the (remaining) RA 4 h Houck *et al.*¹ point sources.

Table 1 shows positions accurate to ~ 1 arc s for the four source identifications having new imaging data. These were measured from our CCD frames in relation to secondary positional standards, whose coordinates were in turn determined relative to nearby Smithsonian Astrophysical Observatory (SAO) Catalogue stars on the POSS plates. Figure 1 shows that correspondence with the Houck *et al.*¹ positions is generally reasonable (the one exception being 0422+009). In the lower part of Table 1, we give positions for other galaxies marked in Fig. 1.

The four galaxy identifications in Fig. 1 all seem to be members of small clusters, groups, or pairs, and at least two of these objects seem morphologically peculiar and may be interacting with nearby neighbours. This could be an important clue for understanding the extreme IR properties of these spirals. Interestingly, a high fraction of interacting neighbours was also found for the "IR galaxies" studied by Soifer *et al.*⁵.

Photometry

RI photometry (on the Kron-Cousins system) is presented in Table 1 both for the source identifications and for the nearby companions. The photoelectric standardization again followed common procedures². Magnitudes for both the standard stars and program objects were measured using an aperture photometer program at Dominion Astrophysical Observatory, and the estimated photometric accuracy of the data is ~ 0.05 mag. The galaxy magnitudes quoted were found by first calculating brightness within successively larger apertures until either a stable magnitude was reached, or interfering objects were encountered. The latter was a problem only for 0413+122, and for all but the galaxies in this field, the magnitudes in Table 1 can be considered indicative of total magnitudes.

The *R-I* colours of the source identifications, corrected for galactic reddening, range from 0.70 to 0.76. (Reddening corrections have been calculated using van de Hulst curve number 15 interpolated to the effective wavelengths of our filters; and for galactic extinction we adopt the colour excesses listed by Houck *et al.*¹.) Comparison with the *R-I* colours of nearby disk galaxies is hampered by the fact that such photometry appears only rarely⁶⁻⁸, and what does exist must be converted with some uncertainty from the Johnson to Kron-Cousins system⁹. Nevertheless, it seems that the *R-I* colours of our IRAS source counterparts are completely normal. For example, the transformed spiral colours measured by Johnson⁶ range from 0.66 to 0.78. The *RI* colours for the other objects listed in the lower part of Table 2 also do not appear unusual, except perhaps for the rather red colour of galaxy number 3 in the 0404+101 field.

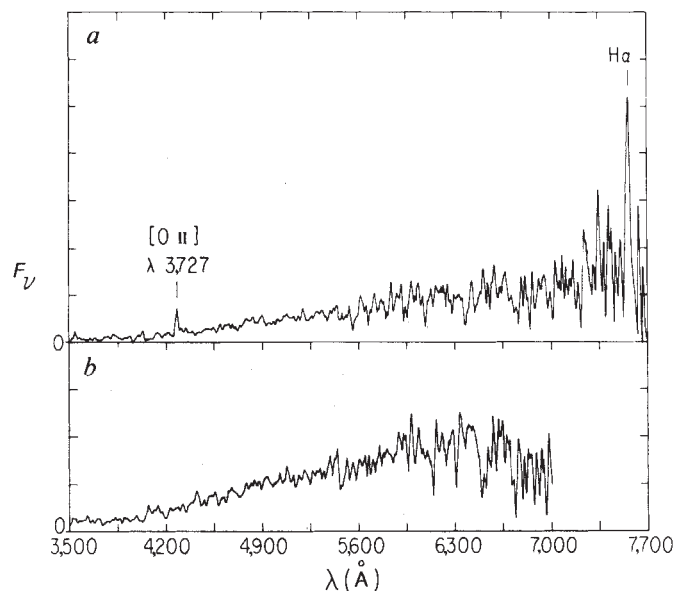


Fig. 3 MMT Reticon spectra of *a*, 0422+009 and *b*, 0425-012. The flux scale is linear. The spectrum in *b* has not been plotted longward of 7,000 Å, where it is severely degraded by noise.

The *R-I* colour primarily measures the old giant population common to all galaxies, and apparently has little sensitivity to other galaxian components. In particular, Johnson's *R-I* colour of 0.66 for the proto-typical starburst galaxy M82 falls within the range of colours covered both by his spirals and ellipticals. (Note also the *k*-correction for *R-I* remains < 0.05 mag for $z < 0.2$; M. Lebofsky, personal communication.) Hence, the *RI* colours provide little insight into the physical nature of the IR sources.

With the present data we can, however, estimate the ratio of luminous output at 80 μ m to that in the blue for comparison with the similar quantity calculated by de Jong *et al.*¹⁰ for Shapley-Ames galaxies and by Soifer *et al.*⁵ for IR galaxies from the IRAS mini-survey. For this, we need to make three corrections: one for galactic extinction, one for internal absorption, and one for conversion from *R* to *B* magnitudes. It is probably the second of these which introduces the greatest uncertainty into the results. The first correction has already been mentioned. For the third correction we adopt $B-R = 1.3$ mag, a value appropriate to face-on spirals in the catalogue of Bothun *et al.*¹¹. (For 0425-012 we additionally assumed an *R-I* value of 0.9 mag.)

The correction for internal absorption (*A*) is more problematic. Given the limited and exotic nature of our data, application

Table 1 Coordinates, colours and fluxes of candidate IRAS galaxies and field galaxies

Name	RA (1950)	Dec (1950)	m_I	$(R-I)$	Aperture diameter (s)	Estimated $m_{B_{0.1}}$	F_{80} (Jy)	$(\nu F_\nu)_{80} / (\nu F_\nu)_B$
I0404+101G1	04 h 04 min 44.85 s	10° 11' 57.2"	18.20	0.96	14.4	19.58	2.38	209
I0413+122G2	04 13 47.30	12 17 38.2	18.27	0.90	13.2	19.59	2.80	251
I0422+009G1	04 22 55.87	00 56 18.2	16.69	0.80	16.8	18.27	1.84	49
I0425-012G1	04 25 11.36	-01 14 39.1	16.47	—	16.8	18.23	1.38	35
Other galaxies								
I0404+101G2	04 04 46.06	10 11 37.7	19.47	0.82	9.6	—	—	—
I0404+101G3	04 04 40.85	10 11 30.8	17.10	1.22	16.8	—	—	—
I0412+085G1	04 12 33.56	08 31 22.5	19.84	—	12.0	—	—	—
I0412+085G2	04 12 33.56	08 31 35.2	19.14	—	13.6	—	—	—
I0412+085G3	04 12 32.93	08 31 44.9	17.74	—	13.6	—	—	—
I0413+122G1	04 13 47.59	12 17 47.4	18.30	0.98	10.8	—	—	—
I0413+122G3	04 13 47.22	12 17 33.0	18.60	0.91	9.6	—	—	—
I0422+009G2	04 22 53.42	00 56 39.3	17.95	0.96	16.8	—	—	—
I0425-012G2	04 25 12.65	-01 13 44.5	17.33	—	12.0	—	—	—
I0425-012G3	04 25 14.72	-01 14 53.2	17.94	—	13.2	—	—	—
I0425-012G4	04 25 14.13	-01 15 25.5	16.89	—	12.0	—	—	—

of any of the standard absorption prescriptions seems difficult. We have instead chosen to adopt $A_R = 0.3$ mag (or $A_B = 0.5$ mag). This value is typical for the 'average' inclined spiral, if we attribute the difference in the mean spiral $B - R$ colour of 1.5 mag from the face-on spiral colour of 1.3 mag in the catalogue of Bothun *et al.*¹¹ as arising from internal reddening. The resulting implied colour excess $E(R - I) \sim 0.1$ mag is not inconsistent with our measured colours, but we stress that because the galaxies studied here may be quite optically thick in dust, any adopted correction must be viewed with caution.

Our estimated corrected B magnitudes for the IRAS source identifications are given in Table 1, together with the (interpolated) 80- μ m fluxes from Houck *et al.*¹, and the resulting $L(80 \mu\text{m})/L(B)$ ratios. The last cover a range of extraordinarily large values, from 35 to 251. We consider the value of 251 for 0413 + 122 an upper limit, though, because both the magnitude listed for the object is a probable underestimate of the total magnitude, and also because the other two galaxies within the IRAS beam may contribute significantly to the IR flux. Even if the flux is equally shared, though, a still impressive lower limit of $L(80 \mu\text{m})/L(B) \sim 80$ would hold for the triplet system. Figure 2 shows the IR-to-blue luminosity ratio plotted against the ratio of flux density at 100–60 μ m for the galaxies studied here and for those from Soifer *et al.*⁵ and de Jong *et al.*¹⁰. As can be seen, only one object from these other surveys falls within the high range of IR-to-blue luminosity ratio encompassed by the present sample. [Comparison with and between the de Jong *et al.*¹⁰ and Soifer *et al.*⁵ results is a little confused by the differing precepts these authors adopt for galactic reddening and internal absorption corrections. For example, applying the procedures of Soifer *et al.*⁵ to M31 yields for this spiral $\log [L(80 \mu\text{m})/L(B)] = -1.09$, a value more in line with other low IR emitting galaxies (see Fig. 2).]

Spectroscopy

Recently, we have obtained spectra for two of the identified sources, 0422 + 009 and 0425 – 012, using the Multiple Mirror Telescope (MMT) and the 'big blue' photon counting Reticon system. Our flat-fielded, sky-subtracted, and flux-calibrated results are presented in Fig. 3. Both objects were observed on two nights (29–30 and 30–31 January 1984) for a combined integration time of 40 min for 0422 + 009 and 30 min for 0425 – 012. The 1×3 arc s slit used yielded an instrumental resolution of 5 Å, although the spectra plotted in Fig. 3 have been smoothed to ~ 20 Å resolution.

Two emission lines are visible in the spectrum of 0422 + 009, which we identify with [O II] $\lambda 3,727$ and H α . These yield a redshift $z = 0.1544 \pm 0.0005$. For a Hubble constant $H_0 = 80 \text{ km s}^{-1} \text{ Mpc}^{-1}$, we obtain an estimated absolute blue magnitude of $M_{B_0} \approx -20.5$, a value sufficiently bright to suggest that the large IR-to-blue luminosity ratio primarily reflects excess IR emission, and not simply great dimming of starlight by dust. At our original instrumental resolution, the [O II] line is unresolved. However, we do appear to resolve H α , and obtain a formal FWHM value of $\sim 600 \text{ km s}^{-1}$, but the uncertainty in this result is large due to the poor signal-to-noise ratio. Perhaps surprisingly, no obvious emission features are present in the spectrum of 0425 – 012; nor is the signal-to-noise ratio adequate to show believable absorption features.

Our spectrum of 0422 + 009 has other notable attributes. First, it does not fit easily into any of the various empirical classification schemes that have been developed for extragalactic line emission regions^{12,13}. In particular, the weakness and/or absence of both H β and [O II] $\lambda 5,007$ is atypical of giant H II region spectra. Nor is there much evidence for the presence of [N II]

$\lambda 6,584$, one of the characteristic lines seen in a low excitation class of objects Heckman calls "liners"¹². The large ratio of H α to [O II] that is seen implies considerable reddening, a not unexpected result. The presence of line emission is itself not so unusual; for instance, about one-third of all nearby bright galaxies are liners, which are found among both spirals and ellipticals¹². However, an intriguing aspect of the 0422 + 009 spectrum involves the large rest frame equivalent widths of [O II] $\lambda 3,727$ (50 Å) and H α (90 Å). Given the object's redshift, if the line-emitting region is confined solely to the nucleus, then even a conservative correction for background continuum flux would yield equivalent widths comparable with those seen in many nearby Seyferts. Clearly, long-slit spectra of high signal-to-noise ratio would help to clarify the nature of the excitation mechanism involved.

Powering the IR emission

The IRAS sources identified here seem to be extreme examples of the IR galaxies discussed by Soifer *et al.*⁵. We now briefly consider what underlying physical process powers the enormous IR output of these systems.

One possibility involves star formation bursts enshrouded by optically thick dust. Indeed, the seemingly high fraction of interacting galaxies found both here and by Soifer *et al.*⁵ may (at least in some cases) provide the triggering mechanism for the outbursts. Further evidence for a thermal rather than non-thermal origin for the IR emission comes from recent 1.5 GHz Very Large Array (VLA) observations by R. Elston (personal communication), who observed but could not detect (with a 0.6 mJy 3- σ upper limit) three IR galaxies from Soifer *et al.*⁵ having $L(80 \mu\text{m})/L(B) \sim 20$.

Rieke *et al.*¹⁴ have presented a detailed starburst model for M82, and scaling their results up to match the properties of the IRAS source 0422 + 009 discussed above leads to some interesting consequences. First, the absolute IR luminosity of 0422 + 009 is ~ 35 times that of M82, and to produce such a flux from star formation, roughly 10% of the stellar mass in 0422 + 009 must participate in the burst. In such a case, however, non-thermal supernova remnant emission ought to be detectable in the radio¹⁵. Furthermore, the implied star formation rate in 0422 + 009 would be $100 M_\odot \text{ yr}^{-1}$. Our measured H α flux of $(15 \pm 5) \times 10^{-15} \text{ erg cm}^{-2} \text{ s}^{-1}$, crudely estimated by normalizing the continuum flux in Fig. 3a to our R -band CCD photometry, is not inconsistent with such a high rate though. In fact, adopting the H α flux calibration of Kennicutt¹⁶ yields an estimated lower limit to the star formation rate of $\sim 15 M_\odot \text{ yr}^{-1}$, and correcting this value upwards for a reasonable 2–3 mag of internal absorption would produce the requisite rate.

One alternative power source might be an embedded active galaxy nucleus, such as a Seyfert nucleus, as proposed by Antonucci and Olszewski¹⁵. Our data provide little constraint on this possibility. However, future spectroscopic and radio observations of robust IR galaxies such as those discussed here should be able to distinguish between the starburst and active nuclei models.

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- Houck, J. R. *et al. Astrophys. J. Lett.* **278**, L63–L66 (1984).
- Aaronson, M., Schommer, R. A. & Olszewski, E. W. *Astrophys. J.* **276**, 221–228 (1984).
- Shanks, T., Stevenson, P. R. F., Fong, R. & MacGillivray, H. T. *Mon. Not. R. astr. Soc.* **206**, 767–800 (1984).
- Low, F. J. *et al. Astrophys. J. Lett.* **278**, L19–L22 (1984).
- Soifer, B. T. *et al. Astrophys. J. Lett.* **278**, L71–L74 (1984).
- Johnson, H. L. *Astrophys. J.* **143**, 187–191 (1966).
- Burkhead, M. S. & Hutter, D. J. *Astr. J.* **86**, 523–537 (1981).
- Wirth, A. *Astr. J.* **86**, 981–988 (1981).

- Bessell, M. S. *Publ. astr. Soc. Pacif.* **91**, 589–607 (1979).
- de Jong, T. *et al. Astrophys. J. Lett.* **278**, L67–L70 (1984).
- Bothun, G. D. *et al. Astrophys. J. Suppl.* (in the press).
- Heckman, T. M. *Astr. Astrophys.* **87**, 152–164 (1980).
- Baldwin, J. A., Phillips, M. M. & Terlevich, R. *Publ. astr. Soc. Pacif.* **93**, 5–19 (1981).
- Rieke, G. H., Lebofsky, M. J., Thompson, R. I., Low, F. J. & Tokunaga, A. T. *Astrophys. J.* **238**, 24–40 (1980).
- Antonucci, R. & Olszewski, E. Preprint (Herzberg Institute of Astrophysics, British Columbia, 1984).
- Kennicutt, R. C. *Astrophys. J.* **277**, 54–67 (1983).

Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells

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The complete 1,210-amino acid sequence of the human epidermal growth factor (EGF) receptor precursor, deduced from cDNA clones derived from placental and A431 carcinoma cells, reveals close similarity between the entire predicted v-erb-B mRNA oncogene product and the receptor transmembrane and cytoplasmic domains. A single transmembrane region of 23 amino acids separates the extracellular EGF binding and cytoplasmic domains. The receptor gene is amplified and apparently rearranged in A431 cells, generating a truncated 2.8-kilobase mRNA which encodes only the extracellular EGF binding domain.

EPIDERMAL growth factor (EGF), a potent mitogenic polypeptide, initiates cellular responses by binding to specific receptors on the surface of target cells (for reviews see refs 1, 2). Like several other growth factors, the binding of EGF to its cell surface receptor triggers a cascade of intracellular events, including induction of a tyrosine kinase activity intrinsic to the EGF receptor¹⁻⁵. Shortly after binding, the EGF-receptor complexes are localized in clathrin-coated regions of the plasma membrane and are then internalized by the cell.^{6,7}

How these events lead to the stimulation of DNA synthesis and cellular proliferation are unknown. The recent observation that the receptors for platelet-derived growth factor (PDGF)^{8,9}, insulin^{10,11} and insulin-like growth factor (IGF-I)¹², are also tyrosine-specific protein kinases suggested that tyrosine phosphorylation might be an important early step in triggering proliferation. The tyrosine-specific protein kinase activity of several retroviral transforming proteins (reviewed in ref. 13) has led many workers to search for a connection between growth factor receptors and transforming proteins. In their analysis of partial protein sequences, Downward *et al.*¹⁴ demonstrated close similarity between the EGF receptor and the avian erythroblastosis virus (AEV) v-erb-B transforming protein¹⁵; evidence was presented suggesting that the viral transforming protein contained only the transmembrane and tyrosine kinase domains of the avian EGF receptor, and lacked most of the extracellular domain responsible for EGF binding. It was proposed that transformation of cells by AEV was the result, at least in part, of expression of a truncated EGF receptor molecule lacking the extracellular control domain¹⁴.

We have now investigated EGF receptor expression in placental and A431 carcinoma cells¹⁶. A431 cells were chosen because they show two unusual features. First, they possess about 10–50 times more EGF receptors on their surface than most other cell types^{16,17}. These high EGF receptor levels seem to be associated with the presence of a chromosomal translocation (M4) involving chromosome seven¹⁸. Second, their mitogenic response to EGF does not correlate with their increased EGF binding capacity. Thus while low concentrations

of EGF stimulate their proliferation, EGF concentrations mitogenic for many cell lines inhibit proliferation of A431 cells^{19,20}. It was therefore of great interest to investigate the genetic basis for the unusual features of this tumour cell line.

The complete amino acid sequence derived from human EGF receptor cDNAs from placental and A431 cells confirms the findings of Downward *et al.*¹⁴, and matches partial amino acid sequences obtained from purified placental and A431 cell EGF receptor protein. Our findings also demonstrate that the EGF receptor gene is amplified in A431 cells. Furthermore, we describe the detection of an aberrant A431-specific chimaeric mRNA molecule which consists of a fragment of EGF receptor coding sequences recombined with sequences of unknown origin and function. This aberrant mRNA encodes only the external EGF binding domain of the receptor and lacks sequences which would encode the transmembrane and cytoplasmic domains. Expression of a truncated domain similar to the erb-B polypeptide found in AEV transformed cells¹⁴ was not detected but its existence at low concentrations cannot be excluded.

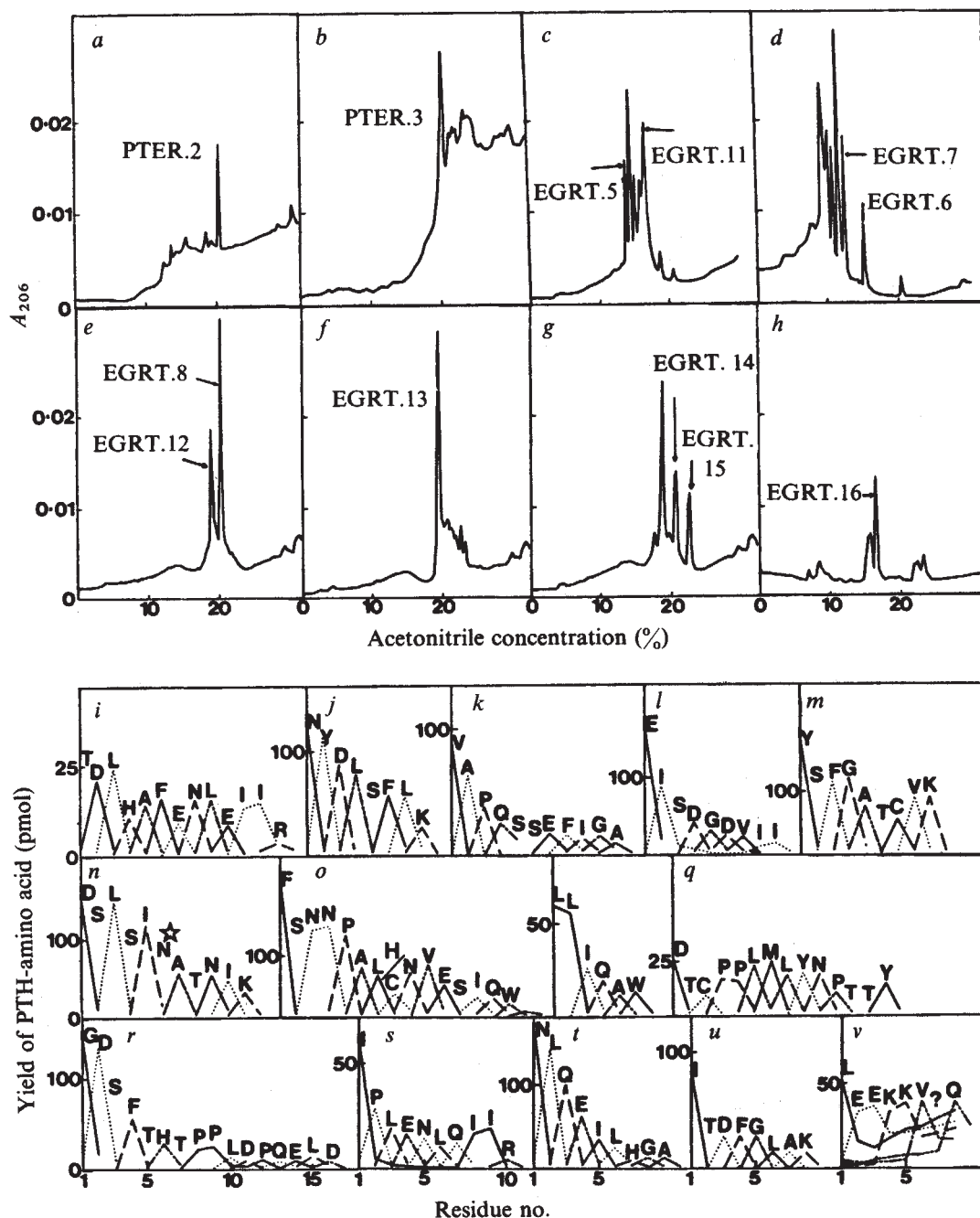
Partial amino acid sequence

Receptor purified by immunoaffinity chromatography followed by gel permeation HPLC in guanidine after reduction and alkylation to cleave disulphide bonds was digested with trypsin and the peptides fractionated by preparative reverse-phase HPLC¹⁴. The purification and partial sequence analysis data of these peptides are shown in Fig. 1. The amino-terminal sequence of the intact receptor was deduced for a short stretch of 8 residues (see Fig. 1v). These partial amino acid sequences were found to be identical (apart from 2 residues) to those subsequently established by nucleotide sequencing of cDNA.

EGF receptor cDNA clones

Oligo(dT)-primed cDNA libraries were prepared from mRNAs derived from human placental tissue and epidermoid carcinoma (A431) cells using the λ gt10 vector system²¹. As analysis of EGF receptor biosynthesis in A431 cells had suggested a polypeptide

Fig. 1 EGF receptor peptide sequence analysis. *a-h*, Purification of peptides from the EGF receptor for sequence analysis. EGF receptor was purified from human placenta (*a*, *b*) or A431 human epidermoid carcinoma cells (*c-h*) using monoclonal antibody R1 (ref. 42); it was then fully reduced and alkylated, run on a gel permeation column, digested with trypsin and the resulting peptides fractionated by reverse-phase HPLC at pH 2.0, as described previously¹⁴. Fractions corresponding to optical density peaks in the first reverse-phase separation were further purified by reverse-phase HPLC on a Synchropak RPP C₁₈ column (4.6 × 75 mm; Synchrom, Linden, Indiana) equilibrated in 10 mM ammonium acetate buffer, pH 6.5. A linear gradient of 0–45% acetonitrile over 45 min was used to elute peptides, flow rate 1 ml min⁻¹. The absorbance of the eluate was monitored at 206 nm and 0.5 ml fractions were collected. The figure shows the resulting chromatograms when the following fractions from the pH 2.0 fractionation were run: *a*, tryptic peptides from the placental EGF receptor eluting at 19% acetonitrile; *b*, placental peptides eluting at 22% acetonitrile; *c*, tryptic peptides from the A431 cell EGF receptor eluting at 21% acetonitrile; *d-h*, A431 peptides eluting at 18, 24, 26, 28 and 22% acetonitrile, respectively. *i-v*, Sequence analysis of peptides from the EGF receptor. Sequence determination was carried out as described¹⁴. The figure shows the yield of PTH derivatives of amino acids at each cycle of the Edman degradation for the peptides: *i*, PTER.2; *j*, PTER.3; *k*, EGRT.3 (for which the full purification has been described elsewhere¹⁴); *l*, EGRT.5; *m*, EGRT.6; *n*, EGRT.7; *o*, EGRT.8; *p*, EGRT.11; *q*, EGRT.12; *r*, EGRT.13; *s*, EGRT.14; *t*, EGRT.15; *u*, EGRT.16. *v*, The amino-terminal sequence for the whole EGF receptor molecule from A431 cells. The protein was treated with 0.05% SDS before sequencing. The seventh residue could not be assigned. ☆, The sixth residue of EGRT.7 was assigned from amino acid analysis of the peptide; the absence of asparagine-PTH at this sequencer cycle suggests that the residue is glycosylated.



backbone of molecular weight (MW) ~ 138,000 (ref. 22), at least 3,500 base pairs (bp) of coding sequence would be required, thus a sizing step was introduced to enrich for cDNA molecules greater than 2,000 bp long. For screening purposes a 51 bp-long DNA hybridization probe (Fig. 2a) was designed and synthesized²³ on the basis of a preliminary partial amino acid sequence of an A431 EGF receptor cyanogen bromide (CNBr) peptide (EGRC.1)¹⁴. Previous studies have shown that the use of a single long probe for clone screening under conditions of low stringency is often superior to the more commonly used technique which involves screening with pools of short oligonucleotide probes²⁴⁻²⁶.

Initial screening of the placental cDNA library (2 × 10⁵ clones)

yielded seven distinct positive clones as judged by *Eco*RI restriction endonuclease mapping and Southern hybridization²⁷ with our synthetic probe. The longest fragment (1.4 kilobases, kb) of clone λ HER-P3 was sequenced using the dideoxynucleotide chain termination procedure²⁸⁻³⁰. The resulting sequence confirmed the presence of a region of similarity with our probe which, despite two errors in the preliminary determination of the amino terminus of the CNBr peptide, differed at only five positions from the sequence of the cloned cDNA (Fig. 2a, c). A second sequence analysis of this peptide gave the amino-terminal sequence Gly-Asp- (see ref. 14) and reinspection of these data revealed a trace of PTH-Asp at step 1 supporting the sequence found in analysis of cDNA clones. Comparison of the

Fig. 2 EGF receptor cDNA nucleotide sequence and predicted amino acid sequence. *a*, The nucleotide sequence of probes based on the amino acid sequence of a CNBr peptide of A431 EGF receptor. The initial amino acid residue was assumed to be methionine since the peptide was generated by cyanogen bromide cleavage¹⁴. The DNA sequence was designed on the basis of published codon usage frequencies⁵⁷. Two overlapping 30 residue-long oligonucleotides (underlined) from coding and non-coding strands were chemically synthesized²³. Schematic representation of human EGF receptor cDNA clones. Overlapping clones from placenta ("P") and A431 ("A") epidermoid carcinoma cells used in sequence determination and a schematic diagram of the complete cDNA structure. Untranslated sequences are represented by a line, coding sequences are boxed. The white portion represents sequences which encode the signal peptide; hatched regions code for the mature receptor, dark hatched regions are high in cysteine residues (see text). The black region demarcates the coding region for the putative transmembrane domain. Black boxes on the 3' ends of λ clones indicate that these clones were obtained by specific priming (complementary sequence at positions 757-780). *c*, Nucleotide and predicted amino acid sequence of EGF receptor. Nucleotides are numbered at the right; amino acids are numbered throughout. Peptide sequences derived from purified receptor preparations are underlined and labelled according to Fig. 1 (*i-u*). The peptide underlines with a broken line was that used to design a long synthetic DNA probe (see Fig. 2*a*). Sites of potential asparagine-linked glycosylation are overlined; the putative transmembrane region is demarcated by a black bar. The AATAAA box close to the polyadenylated 3' end of the mRNA is underlined.

Methods: *a*, The probe was radiolabelled by first phosphorylating the synthetic 30-mers using T4 polynucleotide kinase (PL Biochemicals) and [γ -³²P]ATP (Amersham, >5,000 Ci mmol⁻¹) followed by annealing the kinased single strands and filling in the complementary sequence using *Escherichia coli* DNA polymerase I (Klenow fragment) with [α -³²P]dCTP and [α -³²P]dTTP (Amersham) yielding probes of 5 $\times$ 10⁶ c.p.m. per μ g specific activity. Hybridization was carried out in conditions published elsewhere²⁴. Filters were washed at 45°C with 0.2 $\times$ SSC, 0.1% SDS. Asterisks denote nucleotides of the synthetic probe which did not match the sequence of the isolated clone. *b*, Cytoplasmic mRNA from total human term placental tissue and A431 cells were isolated in the presence of RNase inhibitors following standard protocols⁵⁸. cDNA synthesis⁵⁹, cloning into a λ vector (λ gt10)²¹, and plaque screening⁶⁰ were carried out according to published procedures. *c*, Nucleotide sequence analysis was carried out by subcloning suitable restriction fragments from our recombinant λ clones into M13-based cloning vector (M13mp10 and M13mp11²⁹) followed by primed DNA synthesis on single-stranded DNA templates in the presence of dideoxynucleoside triphosphates^{28,30}.

HER <i>v-erb-B</i>	536	PECLPQAMNITCTGRGPDN	LCQAHYIDGPHCVKTC	PAGVMGNNNTLVW	KYADAGHVCHL	
			PK F A L D R		NA Q	
HER <i>v-erb-B</i>	596	CHPNCTYGTGPGLEGCTNP	GKIPSTATGMV	GALLLVVALGIGLF	MRRRHIVRKRL	
		R K - ST	AV G C V G	YL		
HER <i>v-erb-B</i>	656	RRLQLERELVEPLTPS	GEPNQALLRLKET	EFKIKVLGSGAGF	TVYKGLWIPEGEKV	
			H V I			
HER <i>v-erb-B</i>	716	IPVAIKELREATSPKAN	KEILDEAYVMASVD	NPHVCRLLGICLT	STVQLITQLMPFGCLL	
					Y	
HER <i>v-erb-B</i>	776	DYVREHKDNIGSQYLL	NWCQIAKGMNYLED	RRLVHRDLAARNVL	VKTPQHVKITDFGLA	
		I	E			
HER <i>v-erb-B</i>	836	KLLGAEKEKYHAEGG	KVPKMALESILHRI	YTHQSDVWSYGV	TWELMTFGSKPYDGIP	
		D				
HER <i>v-erb-B</i>	896	ASEISSILEKGERLP	QPPICTIDVYIMV	KCMIDASRPKREL	IIIESKMARDPQRYL	
		V			A P	
HER <i>v-erb-B</i>	956	VIQGDERMHLPSPT	DSNFYRALMDEED	MDVDVDAEYL	IPQGGFFSSPSTR	PLLSLS
			K T E E I	V H N		
HER <i>v-erb-B</i>	1016	ATSNSTVACIDRNLG	QSCPIKEDSFLQ	RYSSDPTGALT	EDTSIDTFLP	VPYEQSVPK
		ATN - GH VR V		NFL E G A V LM		
HER <i>v-erb-B</i>	1076	RPAG-SVQNPVYHN	QNPAPSR---DPHY	QDPHSTAVGNPEY	LVNTVQPTCVNST	FDSPA
		K STAM QI - FISLT	I KLPM SR NS	D	N SPLAKTV E SP	
HER <i>v-erb-B</i>	1132	HWAQKGSHQISL	NPDPYQDFFPKEA	KPNIGFKGSTAEN	AEYLRVAPQSSE	FIGA
		Y I S N N	L TSCS			

Fig. 3 Comparison of EGF receptor with *v-erb-B* predicted amino acid sequences. Only non-matching amino acids are shown for *v-erb-B*. The bracket on the first line indicates the start of the *v-erb-B* sequence¹⁵. The putative transmembrane region is boxed; the arrow demarcates the autophosphorylated tyrosine residue in pp60^{src} (ref. 61). Asterisks indicate residues conserved between the receptor sequence and several members of the *src* gene family (*src*, *yes*, *fes*, *yes*, *fps*). Hyphens (gaps) have been introduced for optimal alignment. The C-terminal tetrapeptide of the *v-erb-B* sequence derived from the AEV *env* gene is underlined¹⁵.

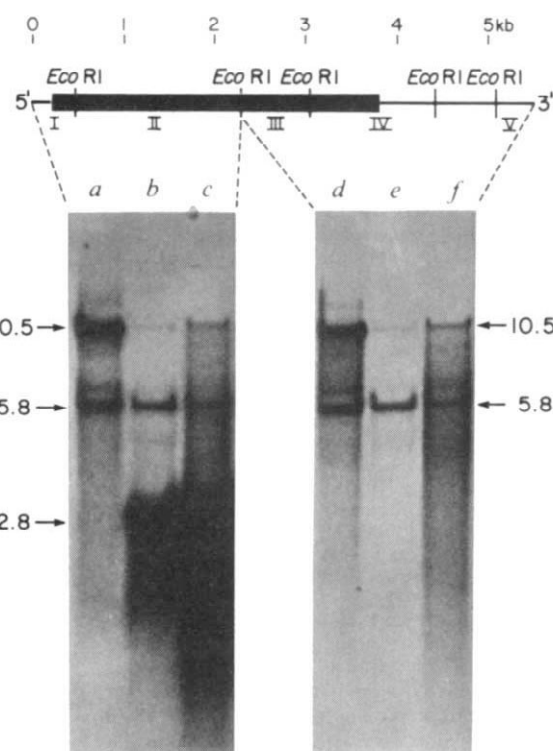


Fig. 4 Northern blot analysis of cytoplasmic placental and A431 mRNAs. Lanes *a* and *d*, placental mRNA; *b* and *e*, A431 mRNA; *c* and *f*, mRNA from a second, independently passaged line of A431 cells. Dashed lines indicate the regions of the schematically depicted cDNA giving rise to the hybridization patterns shown. Numbers on the left and right indicate sizes (in kb) of major mRNA bands. Roman numerals denote *EcoRI* fragments used as hybridization probes (see below).

Methods: A431 cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum in an atmosphere of 10% CO₂-90% air at 37 °C. Cytoplasmic mRNA isolated from frozen placental tissue after pulverization in liquid N₂ and from fresh A431 cells³⁸ was separated (2 µg per lane) on 1% agarose formaldehyde gels and transferred to nitrocellulose filters as described by Lehrach *et al.*³⁶. Rat liver ribosomal RNA (18S = 1,874 nucleotides; 28S = 4,718 nucleotides; I. Wool, personal communication) and 42S vesicular stomatitis virus RNA (~12 kb) were used as size markers. Nitrocellulose filters were hybridized under high-stringency conditions with radiolabelled *EcoRI* fragments (I = 293 bp; II = 1,838 bp; III = 768 bp; IV = 1,373 bp; V = 432 bp) derived from clones λHER-A62 and λHER-A64.

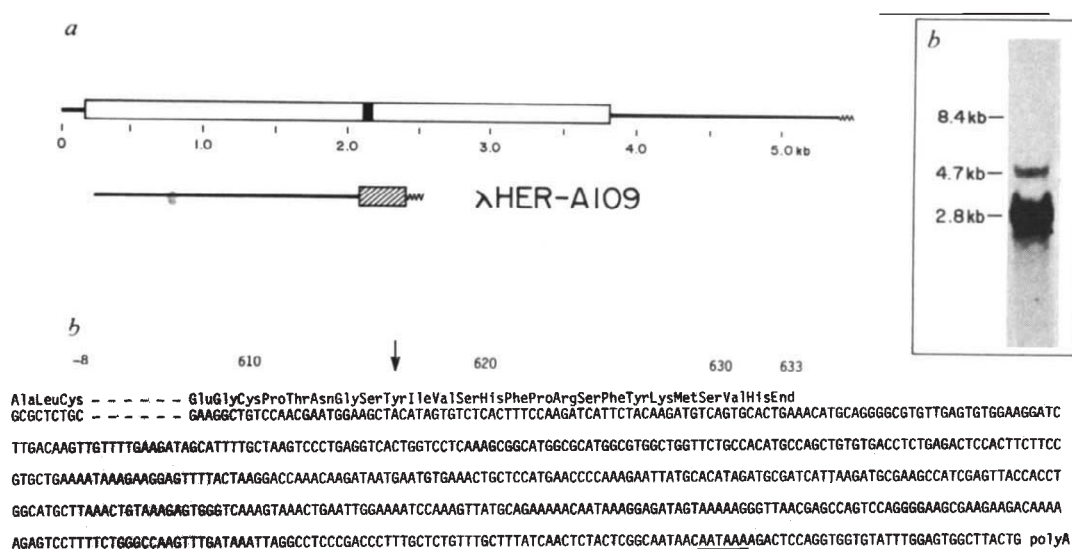
complete sequence of the 2.3-kb cDNA clone λHER-P3 with the nucleotide sequence of the oncogene *v-erb-B*¹⁵ showed that it coded for 243 carboxy-terminal amino acids of the receptor followed by a termination codon and about 1,600 bp of presumably 3'-untranslated sequences lacking a poly(A) homopolymer stretch (see Fig. 2b).

Using the 1.4 kb *EcoRI* fragment of λHER-P3 as a probe, 21 clones were isolated from an A431 cDNA library and characterized by *EcoRI* restriction analysis. Clones λHER-A62 and λHER-A64 were found to contain overlapping DNA fragments, which together spanned a stretch of 5.2 kb (Fig. 2b) of DNA. Complete sequence analysis revealed an open reading frame coding for 1,186 amino acid residues, including carboxy-terminal sequences identical to λHER-P3 and very similar to *v-erb-B* transforming protein. Clone λHER-A62 was found to contain the complete 3'-untranslated region (1,913 bp), as evidenced by the presence of an AATAAA sequence³¹ followed by a poly(A) tail 15 nucleotides downstream.

The amino acid sequences of 13 tryptic peptides as well as those of the 6 previously reported¹⁴ were located within the predicted amino acid sequence (see Figs 1, 2c). Only two differences were detected between the 230 amino acid residues determined by peptide sequencing and those derived from analysis of the cloned cDNA (Cys as opposed to His at position 133). Reinspection of amino acid sequencing data revealed a failure to distinguish between Cys and His in peptide EGRT.8 and to assign amino-terminal Asp in peptide EGRC.1. Remarkably, clone λHER-A64 starts with a nucleotide sequence encoding the amino acid sequence NH₂-Leu-Glu-Gly-Lys-Lys, which is identical to the amino-terminal sequence of the A431 receptor polypeptide (see Figs 1v, 2c).

In order to obtain missing sequences upstream from those which encode the amino-terminus of the mature protein, an A431 cDNA library generated by priming with a 24 nucleotide-long synthetic oligomer (see Fig. 2 legend) was screened with the 5'-most *EcoRI* fragment of λHER-A64. Clone λHER-A21 perfectly matched the sequence of λHER-A64 and extended it 260 bp further upstream. The new sequence contained coding information for an additional 24 predominantly hydrophobic amino acids, including the presumptive initiator methionine. This sequence probably represents the EGF receptor signal peptide which is necessary for transfer of the nascent polypeptide into the lumen of the endoplasmic reticulum. Basic and polar amino acid residues flank the hydrophobic core of the amino-terminal 24-amino acid sequence, a common structural feature of signal peptides. Furthermore, within the 186 bp of 5'-untranslated sequence, an in-frame termination codon is located 36 nucleotides upstream (Fig. 2c), supporting the assignment of residue -24 as the initiation methionine of the 1,210 amino acid-long EGF receptor precursor polypeptide. Placental EGF receptor clones were generated in an analogous priming experiment. Clone λHER-P13 (Fig. 2b) was characterized and matched perfectly the sequence of λHER-A21. These data show

Fig. 5 Structure of an A431 cell-specific cDNA variant. **a**, Schematic map of normal (top) and variant (bottom) cDNA. The solid box represents transmembrane sequences. The black line in the variant cDNA clone λ HER-A109 represents the region of identity with the normal EGF receptor sequence; the cross-hatched box represents a region of unknown origin. ~, Poly (A) tails. **b**, Northern blot analysis using a probe derived from the non-homologous region of variant cDNA clone λ HER-A109. A 260-bp *HpaI-EcoRI* fragment was used as radioactive probe (10^8 c.p.m. μ g $^{-1}$). Blots were exposed for 72 h. Sizes of mRNAs are shown in kb. 28S (4,718 nucleotides) and 18S (1,874 nucleotides) ribosomal RNAs were used as size standards. No hybridization was detected in a parallel experiment with placental mRNA. **c**, Nucleotide sequence of cloned cDNA from λ HER-A109. The start of the cDNA is shown, together with the junction of non-homology to EGF receptor cDNA and the entire non-homologous 3' end.



that both placental and A431 receptors are not synthesized as larger precursor molecules aside from the signal peptide; the unmodified precursor and mature polypeptides have predicted molecular weights of 134,300 and 131,360, respectively. These values are in good agreement with the molecular weight (138,000) determined for EGF receptor synthesized in tunicamycin-treated A431 cells, where *N*-linked glycosylation does not occur²².

Human EGF receptor/*v-erb-B* homology

Partial amino acid sequence analysis of peptides obtained from placental and A431 cell EGF receptor preparations had demonstrated that the EGF receptor matches, in part, the amino acid sequence of the product of the *v-erb-B* oncogene from avian erythroblastosis virus (AEV-H^{14,15}). Availability of the complete EGF receptor sequence derived from cloned cDNA now permits a detailed sequence comparison. Figure 3 shows an alignment of the 604 residue-long *v-erb-B* sequence¹³ with 631 carboxy-terminal amino acids of the human EGF receptor. Extensive amino acid sequence homology is detected over a 376 residue-long core region, beginning at the cytoplasmic junction with the transmembrane domain present in both sequences; 95% of the residues in this region are conserved, which is highly significant considering the evolutionary distance between birds and humans, and therefore probably represent sequences essential for the function of this domain. A 244 residue-long stretch within this region contains 60 residues (24.6%) which are conserved in other oncogenes of the *src* gene family (for clarity, only *src*, *yes* and *fps* are shown in Fig. 3)³². These include the tyrosine which is the substrate for the autophosphorylation reaction of pp60^{src} (refs 33, 34; Fig. 3). The equivalent region of the EGF receptor also retains the amino acid residues thought to be involved in the formation of an ATP binding site^{32,35}. The extent of the sequence conservation is reduced in sequences flanking this 376-amino acid core region until, at residue 600, the *v-erb-B* sequence is fused to the carboxy-terminal tetrapeptide of the AEV *env* protein¹⁵. The EGF receptor sequence continues from the point of divergence at the 3' end for another 32 amino acids. In the light of the sequence identity in the putative tyrosine specific kinase domain, it is surprising that the *erb-B* transforming protein has not been shown to possess kinase activity. This may reflect technical difficulties or could indicate that the carboxy-terminal region, in which there is less similarity between the sequences, may be important for expression of the EGF receptor kinase activity. The complete sequence further sub-

stantiates the hypothesis that *v-erb-B* oncogene sequences originated from cellular sequences which encode the avian EGF receptor¹⁴. As the nucleotide and hence the amino acid sequence of the avian EGF receptor is unknown, the relevance of particular amino acid differences between the human receptor and avian oncogene sequences to the transforming function of the virus remains unclear. The extent of similarity further supports our previous suggestion¹⁴ that other oncogene products that have regions of amino acid sequence similar to *v-erb-B* and the EGF receptor, which form a group known as the *src* gene family, may be derived from cellular sequences encoding other receptors having tyrosine kinase activities such as the PDGF, IGF-I and insulin receptors.

Receptor-related mRNAs

To determine the size of the mRNA encoding the EGF receptor, Northern blot hybridization³⁶ experiments were done using cytoplasmic mRNA isolated from term placenta and from two independently propagated lines of A431 cells. The RNA preparations were probed with various segments of the cloned EGF receptor cDNA. Figure 4 shows that different patterns of hybridization were obtained with probes derived from sequences coding for the 5'-untranslated region and the extracellular domain as compared with probes derived from the cytoplasmic domain and the 3'-untranslated regions. In all experiments, two transcripts of 5.8 and 10.5 kb were detected in both A431 and placental RNA preparations, however the relative amounts of the transcripts differed between placenta and A431 cells, as well as between the two lines of A431 cells. Similarly, two transcripts of 12 and 9 kb have been detected in normal chicken embryo mRNA using *v-erb-B* probes³⁷. These two transcripts could result from transcription of two closely related genes, or, alternatively, represent transcripts of different length from the same gene. Transcription of the mouse α -amylase and dihydrofolate reductase genes, for example, involves usage of multiple poly(A) addition sites, generating mRNAs of variable lengths from a single gene^{38,39}. Alternatively, variable splicing events could generate two mRNAs, perhaps with different function, from a single gene^{40,41}. The variation in the relative levels of the two transcripts in placenta and the two A431 cell lines suggests that some specific mechanism may regulate their relative abundance depending on cell type, physiological condition or developmental state. Further experiments are needed to elucidate this phenomenon.

Probes derived from the 5' half of the cDNA hybridize to a 2.8-kb mRNA present only in A431 cells. Compared with the

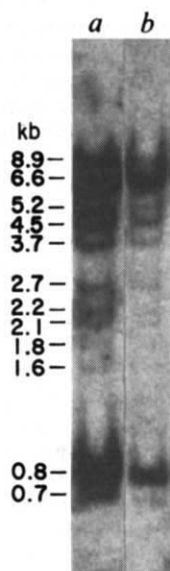


Fig. 6 Southern blot analysis of A431 (a) versus placental (b) DNA. High-molecular weight chromosomal DNA was isolated as described⁵⁸. DNA (10 µg) was digested with excess *Eco*RI (New England Biolabs) then analysed by Southern blot hybridization. A mixture of all *Eco*RI fragments of λHER-A62 and λHER-A64 (as in Fig. 4) was radiolabelled with [α -³²P]dATP and [α -³²P]dCTP (Amersham) by the procedure of Taylor *et al.*⁶². Processed filters were exposed to X-ray film for 5 days at -70 °C using Cronex lighting plus intensifier screens. λ phage DNA was digested with *Eco*RI and *Hind*III was used as a size marker.

two normal mRNAs, this RNA species is overexpressed ~100-fold (Fig. 4b, c). In addition to the normal mRNA bands at 10.5 and 5.8 kb, various fainter bands are observed after long exposure times in both placenta and A431 RNA preparations (see Fig. 4 and data not shown). A 6.1-kb mRNA which is present in both A431 cells and placenta appears to be homologous to all probes used; the same is true for a placenta-specific 13-kb mRNA. Another group of mRNAs from both sources hybridizes with either 5' (1.8 kb) or 3' (8.9 kb) probes. Faint A431-specific hybridization signals are detected with 5' probes at 3.3, 1.65, 1.5 and 1.3 kb in addition to the predominant 2.8-kb mRNA. These bands may represent low-abundance variant transcripts of the same gene, or possibly transcripts of other genes which are structurally related to either the entire EGF receptor or one of its major domains.

Truncated EGF receptor sequences

To characterize in detail the predominant 2.8-kb variant mRNA from A431 cells, an oligo(dT)-primed cDNA bank (>2 kb; ~10⁵ clones) was screened with a 5'-specific EGF receptor cDNA probe (Fig. 4, fragment 1). Of 167 positives, the three clones with the largest inserts were found by restriction enzyme analysis to be related to but distinct from clone λHER-A64. Clone λHER-A109 (2.5 kb) was sequenced and was found to be identical with our EGF receptor cDNA sequence starting at amino acid position -8 in the signal sequence and extending through almost the entire extracellular domain of the receptor. The amino acid sequence identity terminated abruptly, however, at amino acid residue 615, shortly before the transmembrane portion of the normal sequence (see Fig. 5a, c). Downstream from this residue the open reading frame continues for another 18 amino acids, followed by an opal codon (TGA) and a 548-nucleotide 3'-untranslated sequence which includes an AATAAA box 34 nucleotides upstream from the poly(A) addition site. The cDNA (λHER-A109) encodes an approximately 70,000-MW truncated EGF receptor protein which, because it contains a signal peptide but lacks the receptor transmembrane region, should be secreted by A431 cells. Monoclonal antibody R1 (ref. 42), which recognizes the extracellular domain of the EGF receptor, does in fact immunoprecipitate a secreted 115,000-MW glycopeptide, which is thought to be formed by post-translational modification of a 68,000-MW polypeptide²². Analogous truncation of the vesicular stomatitis virus transmembrane glycoprotein by molecular cloning techniques led to secretion of a soluble protein, albeit at a severely reduced rate⁴³.

To establish the identity of this chimaeric cDNA, differential Northern blot hybridization experiments were carried out. Hybridization using a 185-bp *Eco*RI-*Sac*I probe derived from clone λHER-A21, which includes only sequences upstream from the 5' end of the variant clone λHER-A109, resulted in a

pattern identical to that seen in Fig. 4b, c. This finding, in combination with the size and sequence of clone λHER-A109, showed that the 2.8-kb mRNA shares 5' sequences with the 5.8- and 10.5-kb mRNAs. It is not yet clear whether transcription of these mRNAs is under the control of the same regulatory elements or whether sequence rearrangements in the 5'-flanking region of the receptor gene lead to overexpression of the 2.8-kb mRNA. A variant-specific probe (260 bp *Hpa*I-*Eco*I) derived from the 3' end of clone λHER-A109 hybridized to the major A431-specific 2.8-kb band as well as to fainter 4.7- and 8.4-kb bands, but not to the 5.8- and 10.5-kb mRNAs (Fig. 5b). Although the two less abundant (4.7- and 8.4-kb) A431-specific transcripts have the same 3' untranslated sequence as our chimaeric cDNA clone λHER-A109 and therefore could have engendered the cDNA, since neither of them showed homology with various EGF receptor gene sequences, we conclude that our chimaeric cDNA was derived from the 2.8-kb mRNA and that the 4.7- and 8.4-kb mRNAs probably represent transcripts from the parental gene which contain the 3' region of the 2.8-kb mRNA.

Gene amplification

A431 epidermoid carcinoma cells express about 10–50 times more EGF receptors on their surfaces than most other cell types^{16,17}. The remarkably high EGF binding capacity of these cells seems to be correlated with the presence of chromosomal translocations involving chromosome 7 (ref. 18), which is known to carry the EGF receptor gene^{44,45}. In addition to the two translocated fragments of chromosome 7 (M4 and M14), A431 cells contain two copies of the normal chromosome 7 (ref. 18). To investigate further the genetic basis for the high EGF receptor levels in these cells, we compared A431 cell chromosomal DNA with DNA from placenta and other normal tissues. Assuming that the A431 cell chromosomal translocations described by Shimizu and Kondo¹⁸ involve the entire EGF receptor gene, the signal in Southern hybridization experiments for A431 DNA was expected to be twice that detected for DNA from placenta and other human tissues. Figure 6 shows an autoradiograph of a Southern blot hybridization analysis of equal amounts of *Eco*RI-digested A431 (lane a) and placenta (lane b) DNAs. On the basis of densitometric quantification we estimate that the EGF receptor gene is amplified 15–20 times in A431 cells compared with DNA from normal diploid cells. Variable intensities of hybridization signals were observed for different bands within one lane, which may be due to different factors such as efficiency of transfer and binding to nitrocellulose, co-migration of hybridizing DNA fragments and the use of a mixed probe consisting of five DNA fragments of different size (see Fig. 6 legend). The highly selective conditions used in the hybridization experiments excluded cross-hybridization to related but non-identical sequences. Based on more detailed Southern hybridization experiments using various regions of the cDNA to probe separate blots, we estimate that the gene coding for the 5.8-kb EGF receptor mature transcript is greater than 50 kb in size (data not shown).

Our data suggest that various segments of the entire gene are amplified to the same extent. Furthermore it appears that the 8.9-kb hybridization signal obtained with A431 DNA (Fig. 6, lane a) is due to sequence rearrangements, since it is not present in placental and other normal DNAs (Fig. 6 and unpublished results). Additional indications of sequence rearrangements affecting the amplified receptor gene and its flanking regions have been detected in genomic Southern hybridization experiments using the 3' nonhomologous sequence of clone λHER-A109 as a probe (A.U., in preparation).

Discussion

We have presented here for the first time the complete amino acid sequence of a cell surface receptor for a peptide ligand—the mitogen EGF.

Inspection of the receptor's predicted primary sequence reveals a stretch of 23 predominantly hydrophobic amino acids

(residues 622–644) which contains only a single amino acid with a side chain capable of hydrogen bonding. This sequence, in an α -helical configuration, could span the lipid bilayer and the group of basic residues immediately C-terminal (residues 89–101) are likely to interact with the phospholipid head groups in the membrane. It is unlikely that the external domain of the receptor contains regions that cross the lipid bilayer since in A431 cells a truncated receptor resulting from gene rearrangement and consisting of almost the entire external domain is secreted. The cytoplasmic domain may interact with the lipid bilayer but at regions with different structural features from those of the 23-amino acid transmembrane domain. The receptor therefore can be divided into two functional domains—an extracellular one of 621 amino acids and a cytoplasmic domain of 542 amino acids—linked by a short transmembrane region.

The extracellular domain would contain the EGF-binding site. This domain contains 12 of the 16 possible sites for asparagine-linked glycosylation (Asn-X-Ser or Asn-X-Thr). It is likely that the majority of these sites are indeed modified since Mayes and Waterfield²² have shown that 11 sites are glycosylated in the precursor of the secreted external domain of the molecule.

A particularly striking feature of the receptor concerns the distribution of cysteine residues between the extracellular and cytoplasmic domains. The cytoplasmic domain of 542 amino acids contains nine cysteine residues (~2%), a value well within the range of most cytoplasmic proteins. The extracellular domain of 621 amino acids, however, contains 51 cysteine residues, most of which are concentrated within two regions of ~170 amino acids located at positions 134–313 and 446–612 (see Fig. 2c). Alignment of these cysteine-rich sequences with one another reveals similarities in the spacing of the individual cysteine residues, possibly reflecting the involvement of these residues in the formation of two repeated structures and a common evolutionary origin by gene duplication for this part of the receptor. We do not yet know whether these cysteine residues are all disulphide-bonded.

The cytoplasmic domain undoubtedly contains the tyrosine-specific protein kinase activity and has the stretch of amino acid sequence (approximately residues 690–940) that is shared by transforming proteins of the *src* family.

The question remains, how do the domains function in signal transduction across the membrane? If, as we suggest, the EGF receptor polypeptide crosses the membrane only once, then a signal must be transduced through this region unless two or more receptor molecules are involved or an additional polypeptide(s) mediates the response to EGF. What could be the nature of the signal? Since EGF induces rapid changes in ion movements³ it is possible that the receptor itself could function as a channel. Very little is known about structure–function relationships for such channels except in the case of bacteriorhodopsin⁴⁶ and the acetylcholine receptor⁴⁷. For both these proteins it seems that multiple transmembrane segments formed from either a single polypeptide in the case of bacteriorhodopsin or from multiple subunits in the case of the acetylcholine receptor contribute to channel function. Inspection of the putative EGF receptor transmembrane sequence suggests that, even if ligand-induced aggregation brought several transmembrane segments into close apposition, the sequence itself does not contain any amino acids with side chains that could be involved in ion or proton translocation.

An alternative hypothesis is that an EGF-induced conformational change in the external domain is transmitted to the cytoplasmic domain, perhaps as a result of movement of the transmembrane domain but more likely involving receptor aggregation. EGF will induce receptor clustering^{48–50} and antibody cross-linking can mimic the effects of the growth factor^{51,52}. Such clustering of extracellular domains would necessarily bring the corresponding cytoplasmic domains into close proximity, allowing inter-receptor interactions which might include stimulation of tyrosine kinase activity resulting in autophosphorylation. The experimental evidence cannot exclude a mechanism

where a receptor molecule bearing bound ligand interacts with other polypeptides which transduce the transmembrane signal and hence stimulate kinase or other activities indirectly. However it is important to note that EGF can stimulate tyrosine kinase activity of highly purified EGF receptor (ref. 53 and Y.Y., in preparation).

Comparison of receptor gene expression patterns in normal placental tissue and the tumour cell line A431 has revealed distinct differences both at the transcriptional and at the gene structural level. We have shown that the EGF receptor is encoded by a 5.8-kb mRNA in both placental and A431 cells. We also detect a 10.5-kb mRNA which may be a transcriptional variant derived from the same gene, perhaps carrying a longer 3'-untranslated region, analogous to other genes^{38,39}. Both the 5.8- and 10.5-kb mRNAs are expressed at variable but similar levels in both cells, which is remarkable because A431 cells are known to possess 10–50-fold more cell surface EGF-binding sites and associated tyrosine kinase activity than most other cell types^{16,17}. The abnormality we detect instead is that A431 cells synthesize a 2.8-kb mRNA at levels 100-fold greater than that of the larger mRNA species. This mRNA encodes a 70,000-MW secreted polypeptide representing almost the entire extracellular EGF binding domain of the receptor²². Since this truncated receptor is secreted²², it cannot account for the observed overexpression of EGF receptors detected by increased EGF binding capacity¹, monoclonal antibody binding⁴², immunoprecipitation of tyrosine kinase activity^{4,5} and receptor protein quantification as observed on SDS-polyacrylamide gel analysis of these cells¹⁴.

Thus the important question of the mechanism for the dramatically increased EGF receptor levels in A431 cells remains unsolved. We have found that the EGF receptor gene is amplified 15–20-fold in A431 cells, yet amplification does not lead to concomitant increased synthesis of the 5.8- and 10.5-kb mRNAs. A431 cells contain two normal copies of chromosome seven and two chromosomes bearing a chromosome seven translocation¹⁸. Others have observed that gene amplification events are often associated with chromosomal translocations^{54,55}, and Shimizu and Kondo have shown that the overproduction of EGF receptor in A431 cells is genetically linked to the chromosome seven translocation (M4)¹⁸. We propose that the 5.8-kb and 10.5-kb mRNAs are derived from the two normal copies of chromosome seven, while the novel overexpressed 2.8-kb mRNA is derived from the amplified gene copies. Our results do not explain the link between high levels of intact receptors with chromosome 7 translocation.

Amplification of the EGF receptor gene in A431 cells could have occurred intrachromosomally or by generation of acentric extrachromosomal elements (so-called double minute chromosomes). It is possible that the sequence rearrangements we detected (A.U., in preparation) occurred during or after the translocation process and created an altered copy of the EGF receptor gene, leading to truncation and moderate overexpression, before amplification. Alternatively, sequence alterations could have occurred during gene amplification, resulting in overexpression of a single aberrant gene. Detailed analysis of the EGF receptor gene and flanking sequences from normal and A431 cells will be necessary to determine whether the occurrence of the amplified gene generates a truncated mRNA and why this mRNA is overexpressed.

It is also not clear from these results why A431 cells are uniquely unresponsive to the amounts of growth factor which are usually mitogenic for fibroblasts^{19,20,56}.

The finding that A431 vulval carcinoma cells produce a truncated polypeptide which is virtually identical to the external domain of the receptor is particularly intriguing in view of our earlier observation that the *v-erb-B* oncogene encodes what seems to be a truncated avian receptor polypeptide corresponding to the transmembrane and cytoplasmic domains¹⁴. We cannot yet exclude the presence, at a low level, in A431 cells of an mRNA which could encode such a cytoplasmic membrane-associated domain. It seems likely that the A431 2.8-kb mRNA and the secreted EGF receptor external domain are unique to

A431 cells and do not provide any clues to the neoplastic origin or character of this cell line. Since the A431 cell line—and the many variants of this cell line which must now exist (see Fig. 5)—has a multitude of defects exemplified by its 78 chromosomes¹⁸, it is clearly best to regard this cell line with caution when exploring the mechanism of action of EGF or in characterizing changes which relate in a meaningful way to neoplasia.

The structural studies described here provide a basis from which to probe other cell lines and primary tumour tissue for alterations in the EGF receptor, its gene, or its transcription pattern.

After this manuscript was completed two publications related

to the subject appeared. It was shown that A431 cells produce a 105,000-MW protein related to the external domain of the molecule⁶³, as previously reported by Mayes and Waterfield²², and that amplification and altered expression of the EGF receptor gene occurs in A431 cells⁶⁴.

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1. Carpenter, G. *Handbk exp. Pharmac.* **57**, 89–132 (1981).
2. Guroff, G. (ed.) *Growth and Maturation Factors* Vol. 1 (Wiley, New York, 1983).
3. Rozengurt, E. *Molec. Biol. Med.* **1**, 169–181 (1983).
4. Cohen, S., Carpenter, G. & King, L. Jr *J. biol. Chem.* **255**, 4834–4842 (1980).
5. Ushiro, H. & Cohen, S. *J. biol. Chem.* **255**, 8363–8365 (1980).
6. Carpenter, G. & Cohen, S. *J. Cell Biol.* **71**, 154–171 (1976).
7. Schlessinger, J. et al. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2659–2663 (1978).
8. Pike, L. J. et al. *J. biol. Chem.* **258**, 9282–9290 (1983).
9. Heldin, C.-H., Ek, B. & Ronnstrand, L. *J. biol. Chem.* **258**, 10054–10061 (1983).
10. Kasuga, M. et al. *Nature* **298**, 667–669 (1982).
11. Roth, R. A. & Cassell, D. J. *Science* **219**, 299–301 (1983).
12. Jacobs, S. et al. *J. biol. Chem.* **258**, 9581–9584 (1983).
13. Bishop, J. M. A. *Rev. Biochem.* **52**, 301–354 (1983).
14. Downward, J. et al. *Nature* **307**, 521–527 (1984).
15. Yamamoto, T. et al. *Cell* **35**, 71–78 (1983).
16. Fribanc, R. N., De Larco, J. E. & Todaro, G. J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 565–569 (1977).
17. Wrann, M. M. & Fox, C. F. *J. biol. Chem.* **254**, 8083–8086 (1979).
18. Shimizu, N. & Kondo, I. *Cytogenet. Cell Genet.* **32**, 316 (1982).
19. Gill, G. N. & Lazar, C. S. *Nature* **293**, 305–307 (1981).
20. Kawamoto, T. et al. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1337–1341 (1983).
21. Huynh, T., Young, R. & Davis, R. in *Practical Approaches in Biochemistry* (ed. Grover, D.) (IRL, Oxford, 1984).
22. Mayes, E. L. V. & Waterfield, M. D. *EMBO J.* **3**, 531–537 (1984).
23. Crea, R. & Horn, T. *Nucleic Acids Res.* **8**, 2331–2348 (1980).
24. Ullrich, A., Berman, C. H., Dull, T. J., Gray, A. & Lee, J. M. *EMBO J.* **3**, 361–364 (1984).
25. Anderson, S. & Kingston, I. B. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6836–6842 (1983).
26. Jaye, M. et al. *Nucleic Acids Res.* **11**, 2325–2335 (1983).
27. Southern, E. J. *molec. Biol.* **98**, 503–517 (1975).
28. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
29. Messing, J. & Vieira, J. *Gene* **19**, 269–276 (1982).
30. Messing, J., Crea, R. & Seeburg, P. H. *Nucleic Acids Res.* **9**, 309–321 (1981).
31. Proudfoot, N. J. & Brownlee, G. G. *Nature* **263**, 211–214 (1976).
32. Privalsky, M. L., Ralston, R. & Bishop, J. M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 704–707 (1984).
33. Levinson, A. D., Oppermann, H., Varmus, H. E. & Bishop, J. M. *J. biol. Chem.* **255**, 11973–11980 (1980).
34. Erikson, R. L., Collett, M. S., Erikson, E. & Purchio, A. F. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6260–6264 (1979).
35. Barker, W. C. & Dayhoff, M. D. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2836–2839 (1982).
36. Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, H. *Biochemistry* **16**, 4743–4751 (1977).
37. Vennstrom, B. & Bishop, J. M. *Cell* **28**, 135–143 (1982).
38. Tosi, M., Young, R. A., Hagenbuchle, O. & Schibler, U. *Nucleic Acids Res.* **9**, 2313–2323 (1981).
39. Setzer, D. R., McGrogan, M., Nunberg, J. H. & Schimke, R. T. *Cell* **22**, 361–370 (1980).
40. McGrath, J. P. et al. *Nature* **304**, 501–506 (1983).
41. Amara, F. G. et al. *Nature* **298**, 240–244 (1982).
42. Waterfield, M. D. et al. *J. cell. Biochem.* **210**, 149–161 (1982).
43. Rose, J. K. & Bergmann, J. E. *Cell* **30**, 753–757 (1982).
44. Shimizu, N., Behzadian, M. A. & Shimizu, Y. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3600–3604 (1980).
45. Davies, R. L., Grosse, V. A., Kucherlapati, R. & Bothwell, M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4188–4192 (1980).
46. Engelman, D. M., Henderson, R., Mchachlen, A. D. & Wallace, B. A. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2023–2027 (1980).
47. Noda, M. et al. *Nature* **302**, 528–532 (1983).
48. Zidovetzki, R., Yarden, Y., Schlessinger, J. & Jovin, T. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6981–6985 (1981).
49. Haigler, H. T., McKanna, J. A. & Cohen, S. *J. Cell Biol.* **81**, 382–395 (1979).
50. Schechter, Y. S., Hernaez, L., Schlessinger, J. & Cuatrecasas, P. *Nature* **278**, 835–837 (1979).
51. Schreiber, A. B. et al. *J. biol. Chem.* **258**, 846–853 (1983).
52. Schreiber, A. B. et al. *Proc. natn. Acad. Sci. U.S.A.* **78**, 7535–7539 (1981).
53. Parker, P. J. et al. *J. biol. Chem.* (in press).
54. Wahl, G. M., Vitto, L., Padgett, R. A. & Stark, G. R. *Molec. cell. Biol.* **2**, 308–319 (1982).
55. Wahl, G. M., Saint Vincent, B. R. & De Rose, M. L. *Nature* **307**, 516–520 (1984).
56. Barnes, D. W. J. *Cell Biol.* **93**, 1–4 (1982).
57. Grantham, R., Gautier, C., Gouy, M., Jacobzone, M. & Mercier, R. *Nucleic Acids Res.* **9**, 43–74 (1981).
58. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
59. Gray, A., Dull, T. J. & Ullrich, A. *Nature* **303**, 722–725 (1983).
60. Benton, W. D. & Davis, R. W. *Science* **196**, 180–182 (1977).
61. Smart, J. E. et al. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6013–6017 (1981).
62. Taylor, J. M., Illmensee, R. & Summers, J. *Biochim. biophys. Acta* **4**, 324–330 (1976).
63. Weber, W. et al. *Science* **224**, 294–297 (1984).
64. Merlino, G. T. et al. *Science* **224**, 417–419 (1984).

Conservation and change in the DNA sequences coding for alcohol dehydrogenase in sibling species of *Drosophila*

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The DNA sequences of the alcohol dehydrogenase (Adh) genes of four very closely related species of Drosophila show that the rates of nucleotide change vary greatly in different functional domains of this gene. A phylogeny of these species based on the Adh sequence data is consistent with that based on polytene chromosome banding patterns.

SPECIES of *Drosophila* are ideal for studies of DNA sequence evolution as we have very detailed knowledge of the phylogenetic relationships within many groups of the genus¹. *Drosophila melanogaster* is an unusual member of the family Drosophilidae—it is one of a very small number of species (8 out of over 2,700) that is virtually cosmopolitan in its geographical distribution. Its closest relative, *Drosophila simulans*, is also cosmopolitan, although until recently it was rare in South-East Asia and Japan^{2,3}. Recent studies of the drosophilid

fauna of Africa have identified six species closely related to *D. melanogaster* and *D. simulans*^{4–9}. These are *Drosophila yakuba* and *Drosophila teissieri*, widely distributed in sub-Saharan Africa; *Drosophila erecta* and *Drosophila orena*, known only from tropical West Africa; and *Drosophila mauritiana* and *Drosophila sechellia*, endemic to the islands of Mauritius and the Seychelles respectively. These eight species form the *melanogaster* species subgroup. Morphologically they are very similar, their male genitalia providing their only reliable distinguishing features. Ecologically the six Afro-tropical species may be more restricted than either *D. melanogaster* or *D. simulans*: for example *D. erecta* breeds almost exclusively on the fallen

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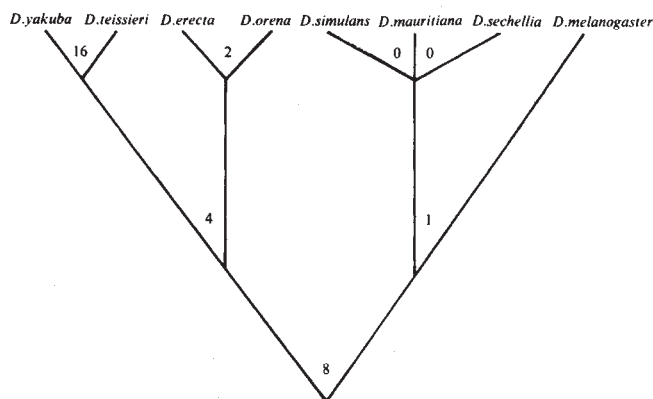


Fig. 1 The relationships among the eight species of the *D. melanogaster* species subgroup based upon their polytene chromosome banding patterns (ref. 10 and F. Lemeunier and M.A., in preparation). Within the *D. melanogaster* complex only one large inversion distinguishes the chromosomes of *melanogaster* itself from those of the other three species. The chromosomes of the members of the *yakuba* complex differ from those of the *melanogaster* complex by at least eight autosomal inversions. The numbers on the diagram indicate the minimum number of autosomal inversions that have accompanied each cladistic event.

fruits of the palm *Pandanus candelabrum* and *D. sechellia* on the fruits of the Rubiaceae shrub *Morinda citrifolia*⁴⁻⁹.

Comparison of the polytene chromosome banding patterns of the *D. melanogaster* subgroup distinguishes two species complexes (ref. 10 and F. Lemeunier and M.A., in preparation). *D. melanogaster*, *D. simulans*, *D. mauritiana* and *D. sechellia* have similar chromosomes, only one large inversion on chromosome 3R distinguishing the chromosomes of *D. melanogaster* from those of the other species of this complex. The chromosomes of the remaining four species differ from those of the *D. melanogaster* complex by at least seven inversions. *D. yakuba*, most dissimilar from *D. melanogaster*, differs by at least 30 inversions¹⁰. Consideration of the inversion events required to derive the polytene chromosome banding patterns among these species gives the tree shown in Fig. 1. This hypothesis is supported by studies of hybridization within the species subgroup¹¹, by studies of the electrophoretic mobilities of certain enzymes¹²⁻¹⁴, by the nature of their mitochondrial, ribosomal and satellite DNAs¹⁵⁻¹⁸ and by the reaction of the species to parasitization by Cynipid wasps¹⁹.

The *Drosophila* Adh gene

Against this background we have sequenced the genes coding for alcohol dehydrogenase (ADH; EC 1.1.1.1) from three of these species and compared them with that of *D. melanogaster* (ref. 20 and H. Haymerle and C. Benyajati, unpublished data). ADH is an abundant protein in all species of the subgroup. In *D. melanogaster* ADH is polymorphic: two electromorphs (ADH^S and ADH^F) are ubiquitous and a third (ADH^{CH-D}) is found in some populations. The amino acid differences between ADH^S, ADH^F and ADH^{CH-D} are known, the three proteins differing in only two positions^{21,22}. Kreitman²⁰ has sequenced the DNA of *Adh* genes from six chromosomes carrying *Adh*^S and five chromosomes carrying *Adh*^F from *D. melanogaster*. Apart from the single base pair change responsible for the lysine to threonine substitution that distinguishes ADH^S from ADH^F, these sequences differ at sites that are 'silent' with respect to the protein.

ADH activity varies during the development of *D. melanogaster*; two peaks of activity are found, the first in mature larvae and the second after eclosion of the adults²³. The gene's transcription pattern differs between larvae and adults, although the two mRNAs encode the same protein²⁴. The transcriptional organization of *Adh* in *D. melanogaster* is summar-

Table 1 ADH amino acid sequence changes

	<i>D. m</i> (ADH-S)	<i>D. m</i> (ADH-F)	<i>D. sim</i>	<i>D. mau</i>	<i>D. ore</i>
2	Ser	Ser	<u>Ala</u>	<u>Ala</u>	<u>Ala</u>
28	Leu	Leu	Leu	<u>Val</u>	<u>Val</u>
51	Lys	Lys	Lys	<u>Gln</u>	Lys
83	Gln	Gln	<u>Lys</u>	<u>Lys</u>	<u>Lys</u>
85	Lys	Lys	Lys	Lys	<u>Thr</u>
100	His	His	His	His	<u>Tyr</u>
193	Lys	<u>Thr</u>	Lys	Lys	Lys
213	Thr	Thr	Thr	Thr	<u>Ile</u>
215	Pro	Pro	Pro	Pro	<u>Ser</u>
231	Gln	Gln	Gln	Gln	<u>Glu</u>
241	Gly	Gly	Gly	<u>Ser</u>	Gly
247	Gln	Gln	Gln	<u>Gln</u>	<u>Lys</u>
249	Thr	Thr	Thr	Thr	<u>Ser</u>

The predicted changes in amino acid sequence of ADH from *D. simulans* (*D. sim*), *D. mauritiana* (*D. mau*) and *D. orena* (*D. ore*) are compared with those of the ADH-S and ADH-F allozymes of *D. melanogaster* (*D. m*). The *D. melanogaster* sequences were directly determined from the proteins²¹ with the exception that an extra tryptophan residue (Trp 252) was identified from the sequence of a partial cDNA clone²⁷. Residues 1-33 are coded for by exon 1, 34-168 by exon 2 and 169-256 by exon 3. Residue 1 is counted as the initiating methionine. Differences from ADH-S are underlined.

ized in Fig. 2. The 'larval' and 'adult' ADH mRNAs differ in their 5' ends. The primary adult transcript initiates 707 base pairs (bp) upstream from the larval; the removal of a 654 base intron from the primary adult transcript gives two mRNAs that differ by 50 bases in size.

Adh sequences in sibling species

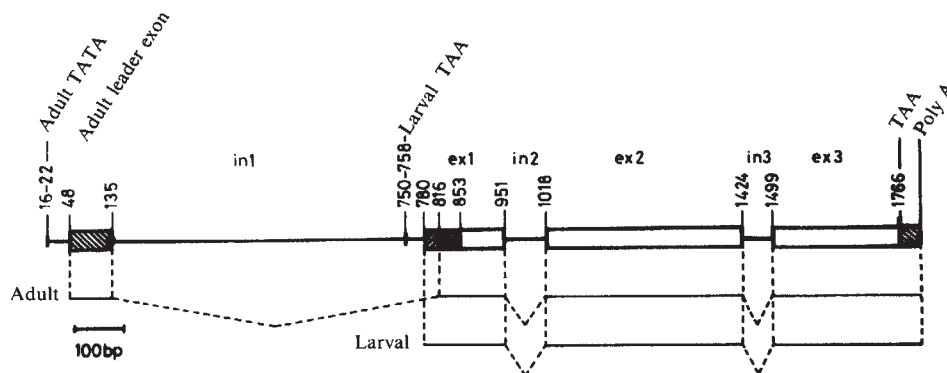
Using the clones of *D. melanogaster* *Adh* isolated by Goldberg²⁵, we isolated the *Adh* genes of *D. mauritiana*, *D. simulans* and *D. orena* (see legend to Fig. 3).

The *Adh* gene sequence from these three species is compared with that of *D. melanogaster* in Fig. 3. The general structure of *Adh* is very similar, suggesting that the overall transcriptional organization is identical in the four species. The coding regions are identical in length. Table 1 summarizes the predicted amino acid changes. The amino acid sequence of the *D. simulans* ADH differs from that of *D. melanogaster* ADH^S by two residues: the N-terminal serine residue of *D. melanogaster* is replaced by an alanine residue in *D. simulans*, and the glutamine to lysine change at residue 83 accounts for the difference in electrophoretic mobility between these enzymes²⁶⁻²⁸. The *D. mauritiana* enzyme is predicted to differ from that of *D. melanogaster* at 5 sites and the *D. orena* enzyme at 10. Within the coding region there are 192 'effectively silent' (synonymous) sites at which a single base substitution cannot result in a coding change²⁰. There are nearly 10 times as many synonymous as coding substitutions (Table 2). Overall, 20.8% of the synonymous, but only 2.2% of the non-synonymous, sites differ.

There are two coding region introns in the *Adh* gene of *D. melanogaster*, of 65 and 70 bp. The genes of the three other species have introns at identical places and of similar lengths, though in the case of *D. orena*, of quite different sequence.

Kreitman²⁰, in his study of 11 alleles of *D. melanogaster* *Adh*, found 38 polymorphisms, of which only two affected sequence length. Both of these were within the 5' intron of the adult transcript (intron 1) and both were substitutions in *Adh*^F alleles that involved a partial duplication of surrounding sequences. Among our sequences, 25 of the 172 sequence variations alter length. Two of the sites of insertion within intron 1 (the 7 bp duplication at position 505 of *D. mauritiana* and the 5 bp insertion at 613 of *D. orena*) are very close to the sites of insertion in *Adh*^F alleles²⁰: the *D. mauritiana* duplication is 1 bp 5' to the *Adh*^F distal insertion and the *D. orena* insertion is 3 bp 5' to the *Adh*^F proximal insertion. The longest deletion is seen in *D. orena*—this species lacks 12 bp immediately distal to the

Fig. 2 The general features of the organization of the *Adh* gene of *D. melanogaster* (from refs 24 and 25). in1, in2 and in3 are the three introns and ex1, ex2 and ex3 the three coding exons. The positions of the nucleotides correspond to those of the consensus sequence shown in Fig. 3.



initiating codon. This deletion may account for the lowered levels of both ADH activity and protein in *D. oreana*, which are about 30% of those of *D. melanogaster*²⁹.

Selective or neutral changes?

The most obvious conclusion from the data is that the rate of nucleotide substitution is higher in noncoding than in coding regions, implying selective constraints against coding region changes. Of the 768 nucleotides in the coding region, 576 (75%) could mutate to give an amino acid substitution. Between *D. melanogaster* and *D. oreana*, 14.6% of the effectively silent sites have changed. We can estimate, therefore, that 84 (14.6% of 576) mutations, affecting approximately one-third of the codons, would have occurred in the coding region. Only 12 (2.2% of codons) of these have survived. This supports Kreitman's²⁰ conclusion that natural selection operates against coding mutations, most of which are likely to be deleterious. On the other hand the effectively silent sites show a far higher level of substitution. There are 192 sites within the coding region at which no nucleotide substitution results in an amino acid change. Forty (20.8%) of these are variable, twice the frequency of change seen in the mRNA 5' noncoding regions.

There is significant heterogeneity in the distribution of nucleotide changes amongst the three sections of the coding region that are split by introns 2 and 3. A 3×2 contingency χ^2 test gives $\chi^2 = 9.64$; $P < 0.01$. The coding region in exon 2 is the most highly conserved and that in exon 3 is the least conserved. Six of the amino acid replacements (out of a total of 12) are in the C-terminal 50 residues of the protein. It has been suggested that the coenzyme (NAD) binding sites of *D. melanogaster* ADH lies within the N-terminal 90 residues of the protein²⁸ and we can speculate that both this site, and the substrate binding site, lie within exon 2.

The rate of change in untranslated leader sequences is similar to that of coding DNA, and about half that seen in the intron sequences (Table 2). A similar result has been found in other studies^{30,31}. This suggests that selective constraints are exerted on noncoding regions of mRNAs. They may be due to functional requirements for transcription and modification of the mRNAs, or to the demands for the interaction of other molecules with mRNA. These demands may constrain either the precise sequences of these untranslated regions or simply their secondary structure.

There is clearly a hierarchy of constraint on change in different parts of the *Adh* sequence, the percentage changes descending in the order: exon silent sites (20.8%), introns (14.1%), untranslated leader sequences (8.4%) and amino acid replacement sites (2.2%). Intron boundaries are also very highly conserved, but we cannot quantitate this as we have no rules for defining the precise limits of those sequences required for correct splicing of the primary transcript.

It is remarkable that silent sites within exons are so much more variable than the intron sequences. This unexpected complexity in the patterns of variation within the gene will be difficult to unravel until we have a better understanding of the function of intron sequences.

Evolution and phylogeny

There have been several studies of the genetic distance between members of the *D. melanogaster* species subgroup based upon the observed distribution of electrophoretic polymorphisms¹²⁻¹⁴. In the Hawaiian picture-winged drosophilids Carson³² has correlated genetic distance with time, using the known age of the Hawaiian Islands. On this basis *D. melanogaster* and *D. simulans* diverged about 0.8 Myr ago, *D. simulans* and *D. mauritiana* 0.4 Myr ago and *D. melanogaster* and *D. oreana* perhaps 2.0 Myr ago (assuming that the genetic distance between *D. erecta* and *D. oreana* is small). However studies of electrophoretic variation within *D. melanogaster* and *D. simulans*³³⁻³⁶ have shown as great a genetic distance between geographically distant populations of *D. melanogaster* as between these species. It is interesting that comparisons of the DNA sequence of *Adh* also shows a similar phenomenon, the amount of variation within *D. melanogaster* is, overall, of the same order of magnitude²⁰.

Table 2 Summary of *Adh* gene sequence changes

	Length (bp)	No. of changes				Total	%
		<i>D. m</i>	<i>D. sim</i>	<i>D. mau</i>	<i>D. ore</i>		
Adult leader	134	3	1	0	9	13	9.7
Larval leader	70	1	0	0	3	4	5.7
Subtotal	204	4	1	0	12	17	8.4
Intron 1	648	16	8	11	44	79	12.2
Intron 2	67	2	2	1	6	11	16.4
Intron 3	70	4	2	3	12	21	30.0
Subtotal	785	22	12	15	62	111	14.1
Total non-coding	989	26	13	15	74	128	12.9
Exon 1 (coding region)	96						
Synonymous	24	0	2	2	6	10	41.7
Replacement	72	1	0	1	1	3	4.2
Exon 2	405						
Synonymous	101	1	1	3	10	15	14.8
Replacement	304	1	1	1	2	4	1.3
Exon 3 (coding region)	267						
Synonymous	67	4	0	2	9	15	22.4
Replacement	200	0	0	1	5	6	3.0
Subtotal							
Synonymous	192	5	3	7	25	40	20.8
Replacement	576	2	0	3	8	13	2.2
Total coding	768	7	3	10	33	53	6.9
Grand total	1757	33	16	25	107	181	
%		1.9	0.9	1.4	6.1	10.3	

The numbers of changes from the consensus sequence are shown for the *Adh* genes of *D. melanogaster* (*D. m*), *D. simulans* (*D. sim*), *D. mauritiana* (*D. mau*) and *D. oreana* (*D. ore*). Additions and deletions of more than one contiguous base are counted as one change.

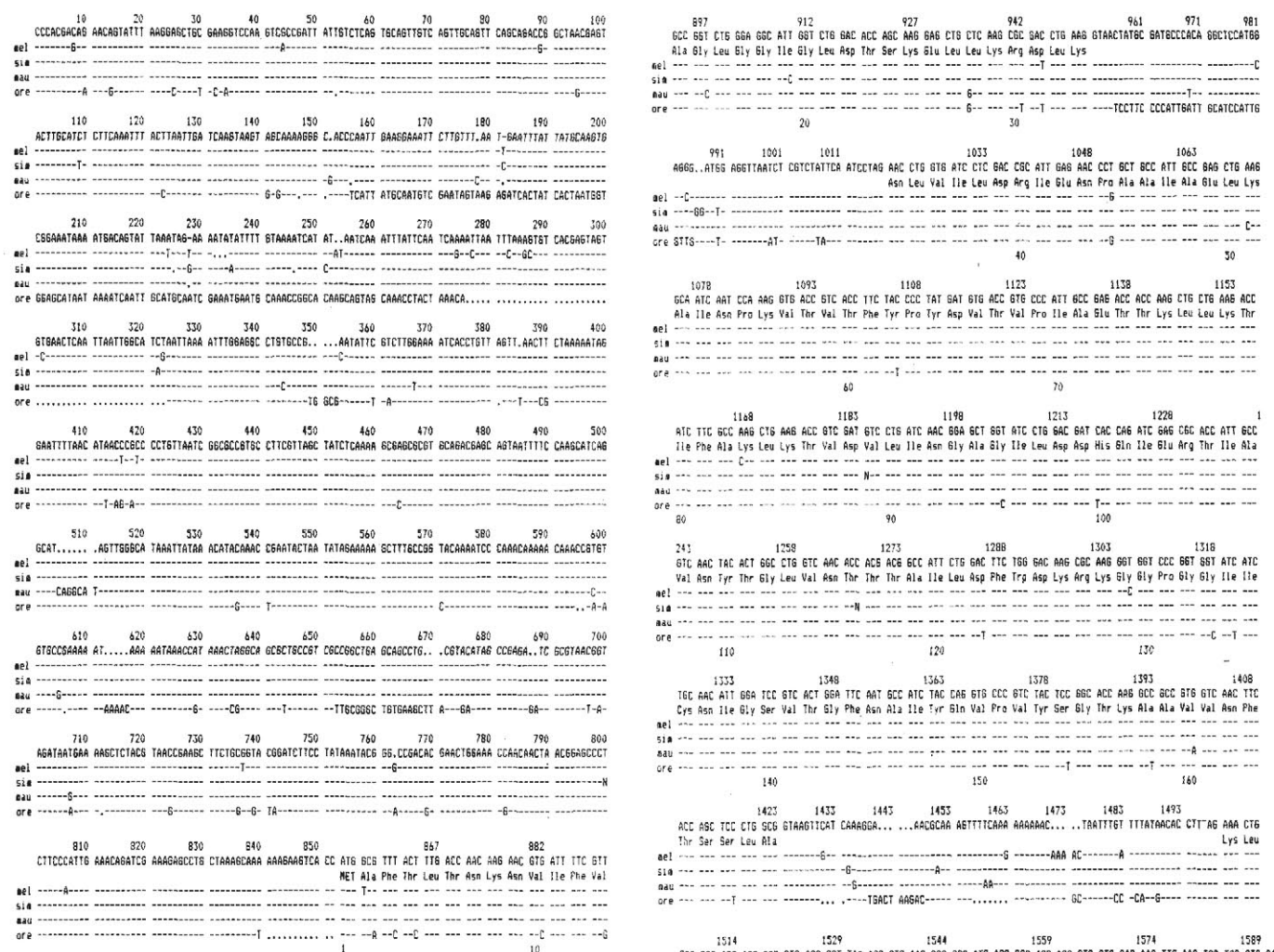


Fig. 3 The DNA sequences of the *Adh* gene of *D. simulans* (sim), *D. mauritiana* (mau) and *D. orena* (ore) and *D. melanogaster* (mel) (Canton-S *Adh*³ allele, H. Haymerle and C. Benyajati, unpublished data) compared with that of a consensus sequence, which represents the most frequent base at each position. The amino acid sequence was derived by translation of the consensus nucleotide sequence. In the DNA sequence hyphens represent a base identical to that of the consensus and dots represent gaps, inserted so as to maximize the similarity of the sequences.

Methods: All sequences were determined by the dideoxyribonucleotide chain terminating method⁴⁸. Genomic libraries of DNA from *D. simulans* (strain C167.4), *D. mauritiana* (strain 163.1) and *D. orena* (strain 188.1) were prepared by cloning in the cosmid Homer 1 (ref. 49) after complete digestion with *EcoRI*. These libraries were probed with a cloned *Adh* gene from *D. melanogaster*, sAC-1 (ref. 25), using the high density colony hybridization procedure⁵⁰. Hybridization of the *Adh* clones of these species to whole genomic DNA, digested with *EcoRI*, confirmed that the fragments cloned were of the same size as the single genomic fragments containing homology to sAC-1. The *D. simulans* sequence was obtained by sonication of the 4.79 kilobase (kb) *EcoRI* genomic fragment of this species that is homologous to sAC-1, and 'shotgun' cloning the fragments into M13mp8 (ref. 51). The *D. mauritiana* sequence was obtained in part by random shotgun clones of the 4.79 kb *EcoRI* fragment homologous to sAC-1 and in part from subclones in M13 made from *HpaII* digestion of the *EcoRI* clone. We assumed, and then verified, that the *HpaII* restriction sites of the 4.79 kb *EcoRI* clones of *D. melanogaster* and *D. mauritiana* are similarly distributed. The final 10 bp of *D. mauritiana* (from the *HpaII* site at 1,756) was not sequenced. The size of the ADH polypeptide of this species, obtained by *in vitro* translation of its mRNA, is indistinguishable from those of the other species of this subgroup²⁹. The *D. orena* sequence was derived from *AluI* and *HaeIII* restriction fragments of a 12 kb *EcoRI* clone (in pAT513) that included the entire *Adh* gene of this species. Subclones in M13 were identified by colony hybridization with a 2.37 kb *SalI*-*XbaI* fragment of sAC-1.

as the difference between single *D. melanogaster* and *D. simulans* alleles.

Miyata³⁷ has shown that in mammals the rate of synonymous codon substitution is rather uniform, at $\sim 5.4 \times 10^{-9}$ per nucleotide per year. If we use this value to estimate the time of divergence of the species of the *D. melanogaster* species subgroup then we obtain times greater than those suggested by the electrophoretic data: 3.9 Myr for *D. melanogaster*-*D. simulans*,

2.9 Myr for *D. simulans*-*D. mauritiana* and 13 Myr for *D. melanogaster*-*D. orena*.

These estimates will be biased by the longer generation time of mammals than *Drosophila*. Moreover, we cannot assume that synonymous substitutions are neutral. Codon usage in *Adh* is nonrandom. This is most clearly seen in a deficit in codons ending in A and T, and a corresponding excess of G and C—a pattern which is also seen in other sequenced *Drosophila* genes

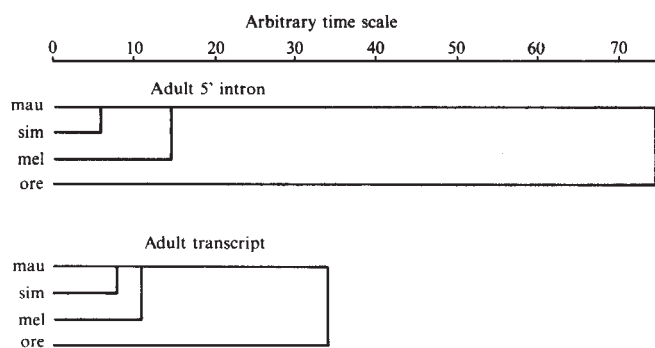


Fig. 4 Trees of the phylogenetic relationships of *D. melanogaster*, *D. simulans*, *D. mauritiana* and *D. oreana* generated by M. J. Bishop's DNAPEQ computer program. The model used by this program makes two assumptions: (1) The genome is under a constant mutational threat, damage being repaired by proofreading functions of DNA polymerases. The model then assumes a constant failure rate of repair at any individual site (μ , which is independent of any previous events at that site) and calculates a probability ($1 - e^{-\mu t}$) that failure will occur over time t . This is termed the exponential failure law. (2) There is an equiprobable chance of replacement by A, C, T or G (allowing for a base to be replaced by itself). The calculation requires that the sequences be aligned for maximum homology. It regards all changes as single base substitutions, and all additions, deletions or replacements are removed. The upper tree is for sequence comparisons of intron 1, the lower tree compares the adult transcripts from their initiation sites with the termination codons. The time scale is arbitrary and could only be transformed to real time were the rates of mutation (μ) known.

(M.A., unpublished data). Nonrandom codon usage is well known in other species (see, for example, ref. 38) and is especially marked in genes whose products are, like ADH in *Drosophila*, abundant^{39,40}. In *Drosophila* the pattern of nonrandomness differs from that of many other eukaryotes due to there being no strong deficit of C-G doublets (M.A., unpublished data), correlating with the very low amount of the modified base 5-methylcytosine in this species⁴¹⁻⁴³. The frequencies of transition and transversion changes among the four sequences are heavily biased towards transitions (Table 3). This is not surprising for the coding regions, as no pyrimidine, and only two purine transitions cause amino acid replacements, but it was unexpected for noncoding regions, which show a 0.75 ratio of transitions:transversions rather than the expected 0.5. In the coding region there is an excess of pyrimidine transitions: 65% of the third positions are pyrimidine, but 82% of the mutations are pyrimidine transitions.

Our data clearly confirm the relationships between the four species of the *D. melanogaster* species subgroup shown in Fig. 2. The sequence of the *Adh* gene of *D. simulans* is closer to that of *Adh*^S of *D. melanogaster* than to *Adh*^F. Kreitman's comparison of *Adh*^S and *Adh*^F alleles shows 17 sites at which a consensus *Adh*^S sequence differs from a consensus *Adh*^F sequence. *D. simulans Adh* resembles *Adh*^S at 14 of these sites (including Lys 193) and resembles *Adh*^F at the other three sites (which are not clustered). As *Adh*^F alleles are relatively rare in the more tropical populations of *D. melanogaster*^{44,45}, these facts point to *Adh*^F as being a more recent allele than *Adh*^S.

Numerical methods of constructing phylogenies have been developed which allow a probabilistic assessment of the relationships among a group of species based upon a defined subset of their attributes⁴⁶. Recently these models have been adapted to make use of nucleotide sequence data (M.J. Bishop & A. E. Friday, personal communication) and a pairwise method of phylogeny analysis has been applied to these data from the *D. melanogaster* species subgroup. We used a program (DNAPEQ) that evaluates a maximum likelihood estimate of divergence times for pairs of DNA sequences. This program uses an exponential failure model of DNA copying error, assum-

Table 3 *Adh* gene transitions and transversions

	Coding	Noncoding
Transitions (t)		
C ↔ T	24	18
G ↔ A	5	15
Sum	29	33
Transversions (v)		
T ↔ G	4	8
T ↔ A	2	12
C ↔ A	9	11
C ↔ G	6	13
Sum	21	44
Ratio (t/v)	1.38	0.75

The numbers are the sums over the four species of the *D. melanogaster* subgroup of single base changes from the consensus sequence shown in Fig. 3.

ing an equiprobable replacement at a site by any of the four bases. DNAPEQ calculates a matrix of pairwise divergence times (on an arbitrary time scale) and these are then used to construct a tree which best fits the pairwise comparisons. Two subsets of the sequence data for the four species were analysed: one was the adult transcript, from its initiation site to the termination codon, and the other was intron 1. The two trees generated are shown in Fig. 4. They are topologically identical, although the estimates of relative divergence times differ. The tree based on the adult transcript sequence places *D. oreana* half the distance from the other species as that based on intron 1. This, of course, is entirely consistent with the different levels of variation seen in these regions of nucleotide sequence. Thus, using independently defined criteria for the construction of cladograms based upon DNA sequence comparisons, the phylogenetic relationships seen in all previous studies are fully confirmed.

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- Throckmorton, L. in *Handbook of Genetics* Vol. 3 (ed. R. C. King) 421-469 (Plenum, New York, 1975).
- Bock, I. R. & Wheeler, M. R. *Univ. Tex. Publ.* 7213, 1-102 (1972).
- Watanabe, T. K. & Kawanishi, M. *Proc. Japan. Acad.* 52, 191-194 (1976).
- Burla, H. *Revue suisse Zool.* 61, 1-218 (1954).
- Tsacas, L. *Bull. Soc. ent. Fr.* 76, 35-45 (1971).
- Tsacas, L. & Lachaise, D. *Ann. Univ. Abidjan Ser. E VII(1)*, 193-211 (1974).
- Tsacas, L. & David, J. *Bull. Soc. ent. Fr.* 79, 42-46 (1974).
- Tsacas, L. & David, J. *Beitr. Ent.* 28, 179-182 (1978).
- Tsacas, L. & Bachli, G. *Rev. fr. Ent.* 3, 147-150 (1981).
- Lemeunier, F. & Ashburner, M. *Proc. R. Soc. B* 196, 275-294 (1976).
- David, J., Lemeunier, F., Tsacas, L. & Bocquet, C. *Ann. Genet.* 17, 235-241 (1974).
- Eisses, K. I., van Dijk, H. & van Delden, W. *Evolution* 33, 1063-1068 (1979).
- Gonzalez, A. M., Cabrera, V. M., Larruga, J. M. & Gullan, A. *Evolution* 36, 517-522 (1982).
- Ohnishi, S., Kawanishi, M. & Watanabe, T. K. *Genetica* 61, 55-63 (1983).
- Fauren, C. M. & Wolstenholme, D. *Nucleic Acids Res.* 8, 2439-2452 (1980).
- Barnes, S. R., Webb, D. A. & Dover, G. A. *Chromosoma* 67, 341-363 (1978).
- Strachen, T., Coen, E., Webb, D. & Dover, G. J. *molec. Biol.* 158, 37-54 (1982).
- Coen, E., Strachen, T. & Dover, G. J. *molec. Biol.* 158, 17-35 (1982).
- Carton, Y. & Kitani, H. *Biol. J. Linn. Soc. Lond.* 16, 227-242 (1981).
- Kreitman, M. *Nature* 304, 412-417 (1983).
- Thatcher, D. R. *Biochem. J.* 187, 875-883 (1980).
- Chambers, G. K., Laver, W. G., Campbell, S. & Gibson, J. B. *Proc. natn. Acad. Sci. U.S.A.* 78, 3103-3107 (1981).
- Ursprung, H., Sofer, W. & Burroughs, N. *Wilhelm Roux Arch. Entomol. Org.* 164, 201-208 (1970).
- Benyajati, C., Spoerel, N., Haymerle, H. & Ashburner, M. *Cell* 33, 125-133 (1983).
- Goldberg, D. *Proc. natn. Acad. Sci. U.S.A.* 77, 5794-5798 (1980).
- David, J. *La Recherche* 89, 482-483 (1978).
- Juan, E. & Gonzalez-Duarte, R. *Biochem. J.* 189, 105-111 (1980).
- Thatcher, D. R. & Sawyer, L. *Biochem. J.* 187, 884-886 (1980).
- Bodmer, M. thesis, Univ. Cambridge (1983).
- Sheppard, H. W. & Gutman, G. A. *Proc. natn. Acad. Sci. U.S.A.* 76, 7864-7868 (1981).
- Miyata, T., Yasunaya, T. & Nishida, T. *Proc. natn. Acad. Sci. U.S.A.* 77, 7324-7332 (1980).
- Carson, H. L. *Nature* 259, 395-396 (1979).

33. Triantaphyllidis, C. D., Panourgias, J. N., Scouras, Z. G. & Ioannidis, G. C. *Genetica* **51**, 227–231 (1980).
34. David, J. R. *Biochem. Genet.* **20**, 747–761 (1982).
35. Singh, R. S., Hickey, D. A. & David, J. *Genetics* **101**, 235–256 (1982).
36. Triantaphyllidis, C. D., Scouras, Z. G., Panourgias, J. N. & Ioannidis, G. C. *Genetica* **58**, 129–136 (1982).
37. Miyata, T. in *Molecular Evolution, Protein Polymorphism and the Neutral Theory* (ed. Kimura, M.) 233–266 (Japan Scientific, Tokyo, 1982).
38. Grantham, R., Gautier, C. & Gouy, M. *Nucleic Acids Res.* **8**, 1893–1912 (1980).
39. Bennetzen, J. & Hall, B. J. *biol. Chem.* **257**, 3026–3031 (1982).
40. Konigsberg, W. & Godson, G. N. *Proc. natn. Acad. Sci. U.S.A.* **80**, 687–691 (1983).
41. Bird, A. *Nucleic Acids Res.* **8**, 1499–1504 (1980).

42. Urieli-Shoval, S., Gruenbaum, Y., Sedat, J. & Razin, A. *FEBS Lett.* **146**, 148–152 (1982).
43. Achwal, C. W., Ganguly, P., Chandra, H. S. *EMBO J.* **3**, 263–266 (1984).
44. Vigue, C. L. & Johnson, F. M. *Biochem. Genet.* **9**, 213–227 (1973).
45. Wilks, A. V., Gibson, J. B., Oakshott, J. G. & Chambers, G. K. *Aust. J. biol. Sci.* **33**, 575–585 (1980).
46. Felsenstein, J. Q. *Rev. Biol.* **57**, 379–404 (1982).
47. Benyajati, C., Wang, N., Reddy, A., Weinberg, E. & Sofer, W. *Nucleic Acids Res.* **8**, 5649–5667 (1980).
48. Sanger, F., Nicklens, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1982).
49. Chia, W., Scott, M. & Rigby, P. W. J. *Nucleic Acids Res.* **10**, 2503–2520 (1982).
50. Hanahan, D. & Meselson, M. *Gene* **10**, 63–67 (1980).
51. Anderson, S. *Nucleic Acids Res.* **9**, 3015–3027 (1981).

LETTERS TO NATURE

The ultraluminous interacting galaxy NGC6240

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In the course of checking preliminary lists of IRAS (Infrared Astronomical Satellite) sources to see whether any of the interacting galaxies that we have been studying¹ were included, we discovered that the position of the IRAS source 1650 + 024PO4 (ref. 2) is within a few arc seconds of one of our programme interacting galaxies, NGC6240. Examination of the Palomar sky survey prints shows that NGC6240 is the only non-stellar object within the 1 arc min IRAS error box for this source. Photometry at 10 and 20 μm from the United Kingdom IR Telescope (UKIRT) confirms that this galaxy is among the most luminous IR galaxies yet discovered. An interaction-induced burst of star formation is the most likely source of the strong IR emission.

Photometry of NGC6240 using UKIRT was obtained on 29 February 1984. We first found the peak in the signal from NGC6240 at K (2.2 μm), and we used this position for the longer wavelength observations. This position coincides within our measurement errors of ~ 2 arc s, with the optical and radio positions discussed below. Our photometric results, obtained in a 4 arc s aperture, are shown in Fig. 1, together with the published preliminary IRAS data, and available optical and radio data. This reveals that the IRAS source is indeed NGC6240.

The nuclear 10- μm luminosity of NGC 6240, inferred from our UKIRT photometry in a 4 arc s aperture, is $1.2 \times 10^{10} L_{\odot}$, based on the measured redshift³ ($7,597 \text{ km s}^{-1}$) and a Hubble constant H_0 of $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$. Integration of the spectrum shown in Fig. 1 yields a bolometric luminosity (L_{bol}) of $2.4 \times 10^{12} L_{\odot}$. Most of this energy is emitted in the IR, with the optical and radio emission contributing only $\sim 10\%$ of the total. This interacting galaxy therefore has an IR luminosity 60 times larger than the archetypal starburst galaxies M82 and NGC253 (ref. 4), for which $L_{\text{bol}} \sim 4 \times 10^{10} L_{\odot}$. Its IR luminosity is an order of magnitude larger than that of the Seyfert galaxy NGC1068 (ref. 5), and comparable with that of Mkn 231 (ref. 6), the most luminous IR galaxy known.

The IR luminosity is most easily understood as arising from a massive burst of star formation, in which the radiation from young, early-type stars is thermalized by dust, and re-radiated in the IR. The quasi-thermal IR spectrum, peaking at $\sim 100 \mu\text{m}$, is typical of that observed in known starburst galaxies. Comparing our 10- and 20- μm fluxes in a 4 arc s aperture with the IRAS photometry at 12.5 and 25 μm in a much larger aperture, suggests that about half of the IR emission comes from a region outside the central 4 arc s. The spatial extent of this emission rules out the possibility that the dust is heated by a single compact object in the nucleus, as has been suggested for some Seyfert galaxies.

However, NGC6240 is clearly not just another starburst galaxy. It is at least an order of magnitude more luminous than typical starburst galaxies, and the recent star formation appears

to be extended over an enormous region in excess of 3 kpc, significantly larger than is generally found for starburst galaxies.

A comprehensive picture of the nature of NGC6240 can be obtained using available optical and radio studies. Photographs presented by Fosbury and Wall⁷ reveal that it is an extremely chaotic interacting galaxy, with a prominent dust lane and three long plumes extending from the central region. Charge-coupled device (CCD) images obtained by Fried and Schulz⁸ show two distinct nuclei separated by ~ 2 arc s. The spectra of Fosbury and Wall, and Fried and Schulz, show intense optical emission lines arising from a region extending over at least 10 arc s. These authors have used the optical emission line intensities to distinguish between different excitation mechanisms (see ref. 9), and they find that the optical emission is characteristic of shock excitation.

NGC6240 is also a very bright radio source with a luminosity at 1,413 MHz (ref. 10) of $4.2 \times 10^{22} \text{ W Hz}^{-1} \text{ Sr}^{-1}$. The Very Large Array (VLA) maps of Condon *et al.*¹⁰ show that the radio emission is coextensive with the region of intense optical line emission. The two nuclei are also clearly resolved at 4,885 MHz. Other bright radio galaxies in the Condon *et al.* sample have an IR (10 μm)-to-radio (1,445 MHz) flux density ratio of ~ 4 . They suggest that this relation can be understood in terms of a starburst model, with the radio emission arising from supernova remnants associated with the starburst. Comparing our 10- μm flux density with the radio flux density for NGC6240 measured by Condon *et al.* for the central 5 arc s gives a ratio of 0.5. Evidently a simple starburst model is inadequate for NGC6240, and an additional energy source for the radio emission is required. Fosbury and Wall⁷ suggest that the strong radio emission in NGC6240 is due to relativistic particles accelerated in the shock fronts. This interpretation appears to be more

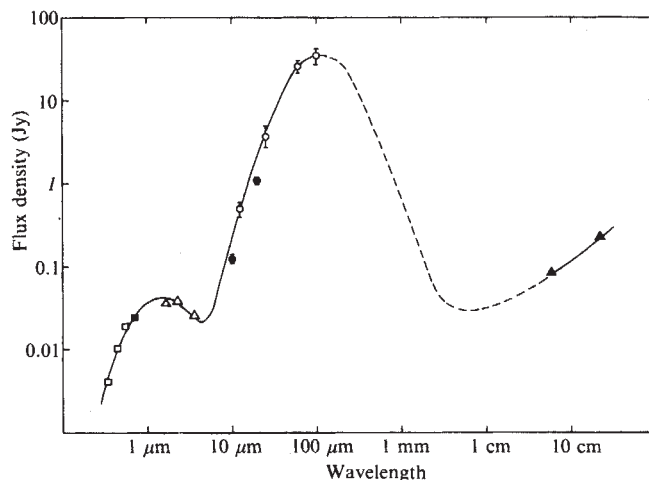


Fig. 1 Continuum spectrum of NGC6240. Data from: ●, this paper; ○, ref. 2; □, ref. 8; ■, ref. 15 and ▲, ref. 10. The dashed interpolation is a Rayleigh-Jeans Spectrum with emissivity proportional to λ^{-1} joined smoothly to the data at longer and shorter wavelengths.

consistent with the small IR-to-radio flux density ratio we find for this galaxy.

The physical picture of NGC6240 which emerges from all of these studies is that of an interaction between two gas-rich galaxies. If the impact parameter is small enough, dynamical friction will be adequate to dissipate the orbital kinetic energy and a merger of the two galaxies will ensue, as discussed by Toomre¹¹ and others. Such interactions produce large non-circular gas motions and infall of material as the colliding galactic nuclei spiral towards each other. The resulting shocks in this material can trigger a massive burst of star formation. NGC6240 seems to be a clear example of this process. The two nuclei, and the strong evidence of shock excitation on large scales, support this model. Furthermore, the thermal character of the IR spectrum, the very large IR luminosity, the spatial extent of the IR emission, and the corresponding spatial extent of the ionized gas are strong circumstantial evidence of a massive burst of star formation, fuelled and triggered by the interaction.

These physical processes and events in NGC6240 are similar to those which we¹ and others (see, for example, ref. 12) are finding in studies of interacting galaxies. NGC6240 is remarkable because it is such a spectacular and energetic example.

There is mounting evidence that interactions are associated with other types of active galactic nuclei^{13,14}. The properties of NGC6240 outlined above demonstrate that this association has physical plausibility. In particular, interaction-induced starbursts are capable of supplying luminosities at least as large as those characteristic of all but the most luminous active galaxies.

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1. Joseph, R. D., Meikle, W. P. S., Robertson, N. A. & Wright, G. S. *Mon. Not. R. astr. Soc.* **208** (in the press).
2. *Nature* **306**, 646 (1983).
3. de Vaucouleurs, G., de Vaucouleurs, A. & Corwin, H. G. *Second Reference Catalog of Bright Galaxies*, 217 (University of Texas Press, 1976).
4. Rieke, G. H., Lebofsky, M. J., Thompson, R. I., Low, F. J. & Tokunaga, A. T. *Astrophys. J.* **238**, 24–40 (1980).
5. Telesco, C. M., Becklin, E. E. & Wynn-Williams, C. G. *Astrophys. J.* (submitted).
6. Rieke, G. H. *Astrophys. J.* **226**, 550–558 (1978).
7. Fosbury, R. A. E. & Wall, J. V. *Mon. Not. R. astr. Soc.* **189**, 79–88 (1979).
8. Fried, J. W. & Schulz, H. *Astr. Astrophys.* **118**, 166–170 (1983).
9. Baldwin, J. A., Phillips, M. M. & Terlevich, R. *Publ. astr. Soc. Pacif.* **93**, 5–19 (1979).
10. Condon, J. J., Condon, M. A., Gislis, G. & Puschell, J. J. *Astrophys. J.* **252**, 102–124 (1982).
11. Toomre, A. in *Evolution of Galaxies and Stellar Populations*, (eds Tinsley, B. M. & Larson, R. B.) 401–426 (Yale University Observatory, 1977).
12. Gehrz, R. D., Sramek, R. A. & Weedman, D. W. *Astrophys. J.* **267**, 551–562 (1983).
13. Balick, B. & Heckman, T. M. *A. Rev. Astr. Astrophys.* **20**, 431–468 (1982).
14. Stockton, A. *Astrophys. J.* **257**, 33–39 (1982).
15. Allen, D. A. *Astrophys. J.* **207**, 367–375 (1976).

Solid white dwarfs as Type I supernova progenitors

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Mass-accreting white dwarfs in close binary systems are generally thought to be Type I supernova (SN I) progenitors. Low-mass ($M_{\text{tot}} \leq 5 M_{\odot}$) binary X-ray sources (also known as Type II sources) equally appear to be the descendants of cataclysmic variables (CV) and thus to have been produced by the collapse of a mass-accreting white dwarf. We point out here that a single process, thermonuclear ignition inside a partially solid, carbon-oxygen white dwarf, may account for the full range of phenomena: from mildly or non-explosive collapses leaving a condensed remnant up to 'slow' to 'fast' SN I outbursts with total disruption of the parent star.

Type I supernovae (SN I) display a high degree of photometric and spectroscopic homogeneity¹. The radioactive decay model^{2,3} successfully accounts for their characteristic light curves. Between 0.2 and 1.0 $M_{\odot}$ of ^{56}Ni should be synthesized in each explosion; the exact values depend on the adopted value of the Hubble constant: $H_0 \sim 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$ would correspond to the lowest masses and would imply a condensed remnant being left by the explosion⁵. Despite the aforementioned homogeneity, some range of variation is detected among the outbursts⁶: 'slow' supernovae show higher peak luminosities, higher velocities in their ejecta and slower declines in their light curves; 'fast' supernovae are dimmer, the velocities of the expanding material are lower and the light curve's decay is faster. Those characteristics are well fitted by Arnett's analytical models⁷. An 'effective diffusion time' τ_m is defined:

$$\tau_m \propto (M_{\text{ej}}^3 / M_{\text{Ni}})^{1/4}$$

where M_{ej} and M_{Ni} are, respectively, the masses of the ejecta and of the synthesized ^{56}Ni . Larger τ_m values mean slower light curve declines. The expansion velocities and the peak luminosities are also related to the above quantities by

$$v_{\text{ej}} \propto (M_{\text{Ni}} / M_{\text{ej}})^{1/2}$$

and

$$L_{\text{peak}} \propto M_{\text{Ni}}$$

The most fashionable model for explaining the outbursts involves central deflagration of a white dwarf in a close binary system, leaving no remnant⁸, but this model implies a 'unique' configuration and does not allow for variation (M_{ej} coincides with M_{tot} and the latter is roughly the Chandrasekhar's mass). Different explosion mechanisms (helium detonation, helium-carbon detonation, and so on) may then be invoked, but their predictions contradict the observations, as almost no intermediate mass elements are ejected in those models. A natural way for fitting the light curves is, however, to assume that M_{ej} is a variable fraction of M_{tot} . Other types of observations, as positron events at the galactic centre^{9,10}, also suggest thermonuclear explosion with relatively small amounts of ejecta¹¹.

Type II galactic X-ray sources (comprising globular cluster sources, galactic bulge sources, Sco X-1 type sources, and X-ray bursters) share the common characteristic of involving a neutron star with a low-mass ($M \leq 1 M_{\odot}$) companion. While the capture mechanism might be a valid explanation for the globular cluster sources, it cannot explain the other kind of sources. Besides, the distribution of X-ray galactic bulge sources and that of the novae in M31 show a remarkable coincidence¹². Collapse of a white dwarf in a cataclysmic variable is the most likely formation mechanism¹³.

We thus have a situation which strongly suggests that mass accreting white dwarfs in close binary systems may at some point collapse and/or explode leaving condensed remnants of various masses: from practically the same mass as the collapsing white dwarf up to zero mass in the case of complete disruption. In contrast, there is almost no model which predicts such a continuous range of behaviour.

The effects of solidification in cooling carbon-oxygen white dwarfs have been pointed out¹⁴. One possibility is that carbon/oxygen separation happens at phase transition: oxygen 'snow' falling to the centre and carbon being rejected to the outer layers. The likely outcomes¹⁵ are either collapse initiated by the electron captures on oxygen at the centre or off-centre carbon ignition. With the lack of a detailed numerical simulation of the liquid/solid phase transition, we consider other alternatives, such as 'percolation' in the solid phase¹⁶: the free energy is lower when the carbon ions imbedded in an oxygen lattice are surrounded by oxygen ions. So, if carbon were even only slightly less abundant than oxygen at the end of the helium burning (as the recent revision of the $^{12}\text{C}(\alpha, \gamma)^{16}\text{O}$ rate suggests¹⁷), the relevant rate for the ignition would be that of $^{12}\text{C} + ^{16}\text{O}$ instead of $^{12}\text{C} + ^{12}\text{C}$. Central ignition would occur when the central density $\rho_c \sim 10^{10} \text{ g cm}^{-3}$ and would also lead to

collapse. But even if carbon were more abundant than oxygen and/or no separation mechanism were operating solidification would still ensure at least partial collapse. This is because when thermonuclear ignition happens in the middle of a solid, the burning propagates by conduction (the successively melted layers only afterwards become convective). The characteristic speeds are between 10^{-4} and 10^{-5} times the local speed of sound¹⁸. Taking into account the new corrections to the electron screening factors¹⁹, the ignition density of the reaction $^{12}\text{C} + ^{12}\text{C}$ becomes $\rho_c \sim 3.5 \times 10^9 \text{ g cm}^{-3}$. For instance, assuming the interior to be solid from the centre out to a radius $5 \times 10^7 \text{ cm}$ (about half the stellar radius), it takes $\sim 500 \text{ s}$ for the conductive burning front to reach the edge of the solid core (in contrast with $\sim 1 \text{ s}$ if we had assumed a convectively-driven front, or $\sim 0.1 \text{ s}$ for a detonation wave). Even at those relatively low ignition densities, the electron captures on the matter in nuclear statistical equilibrium (NSE)²⁰ are fast enough to remove pressure efficiently and start the collapse: $\tau_e = Y_e / \dot{Y}_e \leq 5 \text{ s}$ (where Y_e is the electron mole number). When the burning front reaches the fluid layers, it changes into a convectively-driven front and accelerates.

Calculations were performed assuming central ignition in a white dwarf with a central density of $3 \times 10^9 \text{ g cm}^{-3}$, with a solid core of one-tenth of the total mass, composed of a mixture of equal parts of carbon and oxygen. While the burning propagates through the solid core at a velocity of 2 km s^{-1} hydrostatic equilibrium was assumed. When the burning front reaches the solid/liquid transition, the material between that point and the layer corresponding to $1 M_\odot$ was instantaneously incinerated. Then the hydrodynamical behaviour was followed with an explicit numerical code²¹.

Our results are preliminary because we have extrapolated the approximations used for the matter in NSE to higher densities and lower Y_e than prescribed, and the hydrostatic/hydrodynamic transition should be treated more carefully. In the same way, burning propagation in the solid, as well as in the fluid, layers needs a more detailed treatment. Keeping these restrictions in mind, we conclude that the liquid layers are ejected and the solid/fluid transition sets a lower limit to the mass of the bounded remnant. Due to the low densities of those layers ($\rho \leq 2.35 \times 10^9 \text{ g cm}^{-3}$) the neutron excess of the ejected matter is not too high ($\langle \eta \rangle \sim 3 \times 10^{-3}$).

Thus, the mere solidification of the star's interior appears to determine the implosion of the frozen layers on carbon ignition. The burning front, when reaching the still fluid layers mimics an off-centre ignition. A single parameter (the size of the solid core, resulting from the interplay between the cooling time before mass transfer and the heating effects of the accretion process²²) determines the amount of matter ejected. So, the whole range from mildly explosive collapse (leading to the formation of Type II X-ray sources) to 'fast' and 'slow' SN I might be covered by a single mechanism based on a well understood physical process.

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- Branch, D. et al. *Astrophys. J. Lett.* **252**, L61–L64 (1982).
- Arnett, W. D. *Astrophys. J.* **240**, 105–108 (1980).
- Colgate, S. A., Petschek, A. G. & Kriese, J. T. *Astrophys. J. Lett.* **237**, L81–L85 (1980).
- Axelrod, T. S. thesis, Univ. California, Santa Cruz (1980).
- Wheeler, J. C. *Nature* **302**, 209 (1983).
- Branch, D. *Astrophys. J.* **248**, 1076–1080 (1981).
- Arnett, W. D. in *Supernovae: A Survey of Current Research* (eds M. J. Rees, & Stoneham, R. J.) 221–251 (Reidel, Dordrecht, 1982).
- Wheeler, J. C. in *Supernovae: A Survey of Current Research* (eds Rees M. J. & Stoneham, R. J.) 167–203 (Reidel, Dordrecht, 1982).
- Riegler, G. R. et al. *Astrophys. J. Lett.* **248**, L13–L16 (1981).
- Leventhal, M. & MacCallum, C. J. *Proc. Workshop on the Galactic Center* (eds Riegler, G. R. & Blandford, R. D.) 132–138 (California Institute of Technology, 1982).
- Colgate, S. A. & Petschek, A. G. *Nature* **296**, 804–805 (1982).
- Vader, J. P., Van den Heuvel, E. P. J., Lewin, W. H. G. & Takens, R. J. *Astr. Astrophys.* **113**, 328–335 (1982).
- Van den Heuvel, E. P. J. *Space Sci. Rev.* **30**, 623–642 (1981).
- Canal, R., Isern, J. & Labay, J. *Nature* **296**, 225–226 (1982).
- Isern, J., Labay, J., Hernanz, M. & Canal, R. *Astrophys. J.* **273**, 320–329 (1983).

- Schatzman, E. *Cataclysmic Variables and Related Objects, IAU Colloq.*, No. 72, 149–153 (1983).
- Kettner, K. U. et al. *Z. Phys. A* **308**, 73–84 (1982).
- Buchler, J. R., Colgate, S. A. & Mazurek, T. J. *J. Phys. Suppl.* **41**, C2–159 (1980).
- Ichimaru, S. & Utsumi, K. *Astrophys. J. Lett.* **269**, L51–L55 (1983).
- Epstein, R. E. & Arnett, W. D. *Astrophys. J.* **201**, 202–211 (1975).
- Colgate, S. A. & White, R. H. *Astrophys. J.* **143**, 626–681 (1966).
- Nomoto, K. *Astrophys. J.* **253**, 798–810 (1982).

Topography of the shield volcano, Olympus Mons on Mars

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Olympus Mons, one of the largest known shield volcanoes in the Solar System, covers an area of $>3.2 \times 10^5 \text{ km}^2$ and has a diameter of $>600 \text{ km}$, excluding its vast aureole deposits. The structure is five times larger than the largest shield volcano on the Earth. It is situated on the north-west flank of the Tharsis volcanic region, a broad topographic rise on the martian surface. The volcano has three physical subdivisions: the summit caldera, the terraced upper flanks, and the lower flanks, which terminate in a scarp 2–10 km high that nearly surrounds the structure. A large block of images of the Tharsis region, including Olympus Mons, was obtained by the Viking mission¹. Here we present a topographic map of Olympus Mons, compiled using various combinations of stereo pairs of these images, together with stereoscopic perspective views generated by image processing techniques.

The contour map of Olympus Mons was compiled at a scale of 1:1,000,000 with a contour interval of 200 m, using 91 stereoscopic models of Viking Orbiter photographs². Special photogrammetric techniques were used for this compilation. These techniques had been developed to use the Viking Orbiter photographs, which have extremely narrow field-of-view³, for the systematic mapping of Mars. Three hundred and sixteen control points were produced from a block of 103 photographs using analytical aerotriangulation methods. Digital elevation data were collected from stereo models on the analytical plotter at the time of map compilation. Using techniques of digital image processing⁴ shown in Fig. 2, perspective stereoscopic views were made at various vertical viewing angles from different desired azimuths. The summit caldera and the terraced upper flank are viewed monoscopically from the east and the west in Fig. 2a and b, respectively; the scarps of the lower flanks are viewed stereoscopically from the north and the south-east in Fig. 2c and d.

Previous estimates of the height of Olympus Mons ranged from 17 to 23 km above the surrounding area^{5–12}. A height of 27 km above the Mars topographic datum¹³ is shown on the US Geological Survey 1:25 million scale, global topographic map of Mars¹⁴, which was compiled by synthesizing remotely-sensed data from the Mariner 9 Mars mission and Earth-based radar observations^{15–16}. The newly compiled topographic map (Fig. 1), shows the elevation of Olympus Mons to be 26,400 m above the Mars datum. The volume determination of Olympus Mons calculated for individual segments bounded by contour lines on Fig. 1 is given in Table 1.

Olympus Mons has the general form and slope of a classic terrestrial basaltic shield volcano^{8,17,18}. Major topographic features include a complex caldera at the summit that is $\sim 80 \text{ km}$ across, upper flanks that form a concentric pattern of terraced terrain, and a steep basal scarp. The summit caldera is composed of at least five coalesced collapse craters¹⁹. Concentric terraces on the upper flanks are prominent on the east, south-east, west, and south-west sides²⁰. Typically, the terraces are spaced 15–20 km apart. The unique, nearly continuous, basal scarp rises more than 10 km above the basal plains on the north and north-west, (Fig. 1); on the south-east, the scarp rises 4–5 km

Table 1 Volume determination of Olympus Mons

	Elevation (km)	Volume of segment	Accumulated ($\times 10^6 \text{ km}^3$)	Elevation (km)	Volume of segment	Accumulated ($\times 10^6 \text{ km}^3$)	Elevation (km)	Volume of segment	Accumulated ($\times 10^6 \text{ km}^3$)
Above	26	0.0004	0.0004	17	0.073	0.336	9	0.223	1.553
	25	0.006	0.007	16	0.088	0.424	8	0.236	1.789
	24	0.013	0.020	15	0.103	0.527	7	0.247	2.036
	23	0.022	0.042	14	0.120	0.647	6	0.262	2.298
	22	0.029	0.071	13	0.140	0.787	5	0.296	2.594
	21	0.035	0.106	12	0.158	0.945	4	0.365	2.959
	20	0.043	0.149	11	0.180	1.125	3	0.436	3.395
	19	0.052	0.201	10	0.205	1.330	2	0.467	3.862
	18	0.061	0.263						

Volumes were calculated, using a planimeter, for individual segments bounded by contour lines on the map (Fig. 1). The third column lists cumulative volumes, measured downward from the top in increments of 1 km. Blasius^{7,8} reported a total volume of $2.7 \times 10^6 \text{ km}^3$ for Olympus Mons and Beatty²⁵ reported a volume of $3\text{--}4 \times 10^6 \text{ km}^3$. From the new map (Fig. 1) we calculate a total volume of $2.594 \times 10^6 \text{ km}^3$ for the part of the volcano above the 5-km elevation. Assuming a density of 3.0 g cm^{-3} (ref. 26), we estimate the topographic mass of Olympus Mons to be $7.782 \times 10^{18} \text{ kg}$ above the 5-km elevation. If estimated volumes between elevations of 2 and 5 km are added to this calculation, the total volume above the 2-km contour is $3.862 \times 10^6 \text{ km}^3$. This, in turn, gives a total mass of $11.586 \times 10^{18} \text{ kg}$ above the 2-km elevation.

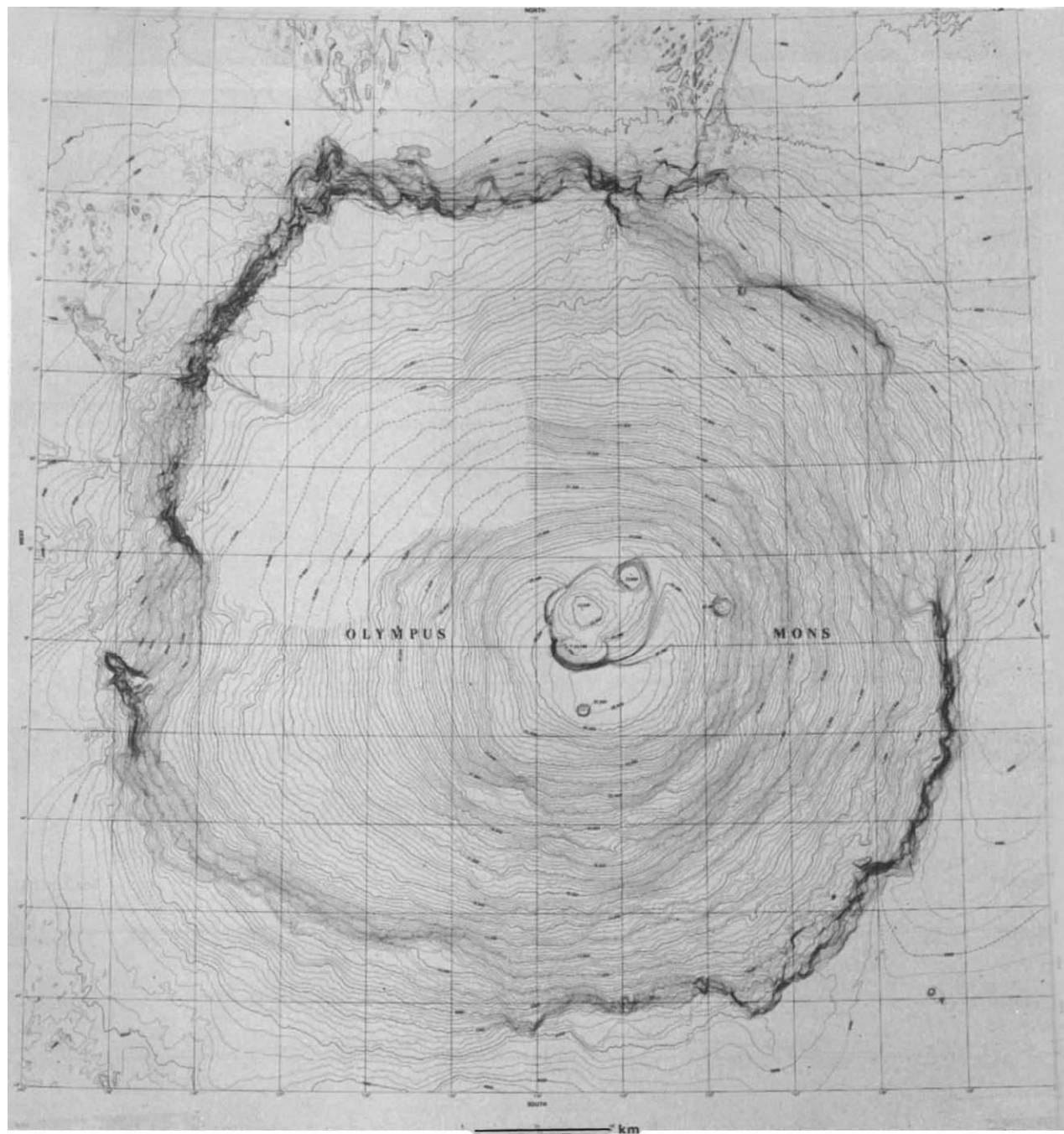


Fig. 1 Topographic map of Olympus Mons. Scale of the map, 1:1,000,000; contour interval, 200 m. Contours and elevations are in metres and are referred to the Mars topographic datum. A transverse Mercator projection was used with the central meridian placed at 134°W long. Dashed contours are approximate.

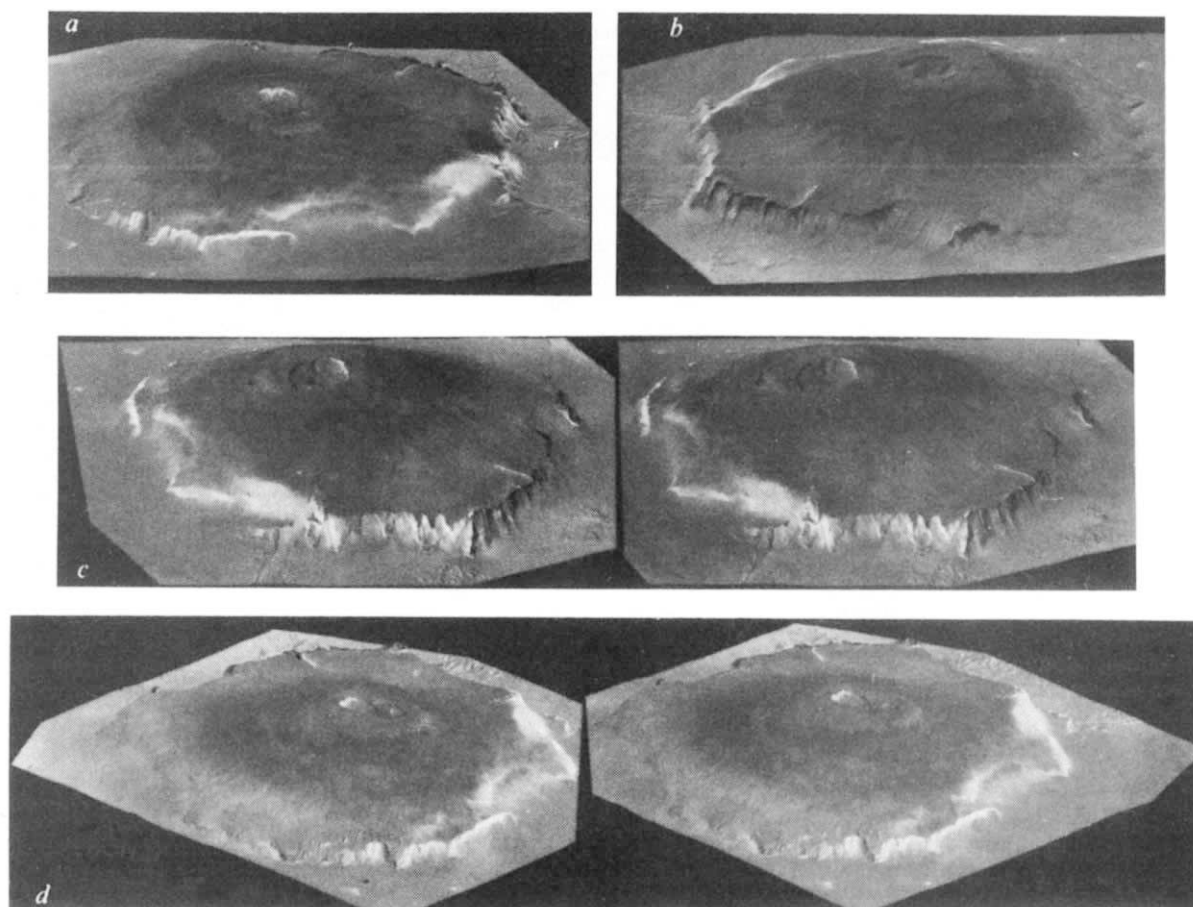


Fig. 2 Perspective views of Olympus Mons. Pictures were generated by digital image processing, from Viking Orbiter photograph 646A28. Projection is controlled by digital elevation data collected at the time of the map compilation (Fig. 1). Viewing angle is 25° from the horizon. Vertical exaggeration, $\times 5$. *a, b*, Monoscopic views from due east and due west; *c* and *d* are stereoscopic views from due north and from the south-east, respectively. Both stereo pairs have 5° separation in azimuth between the left and the right pictures. A stereoscope may be used for stereoscopic viewing.

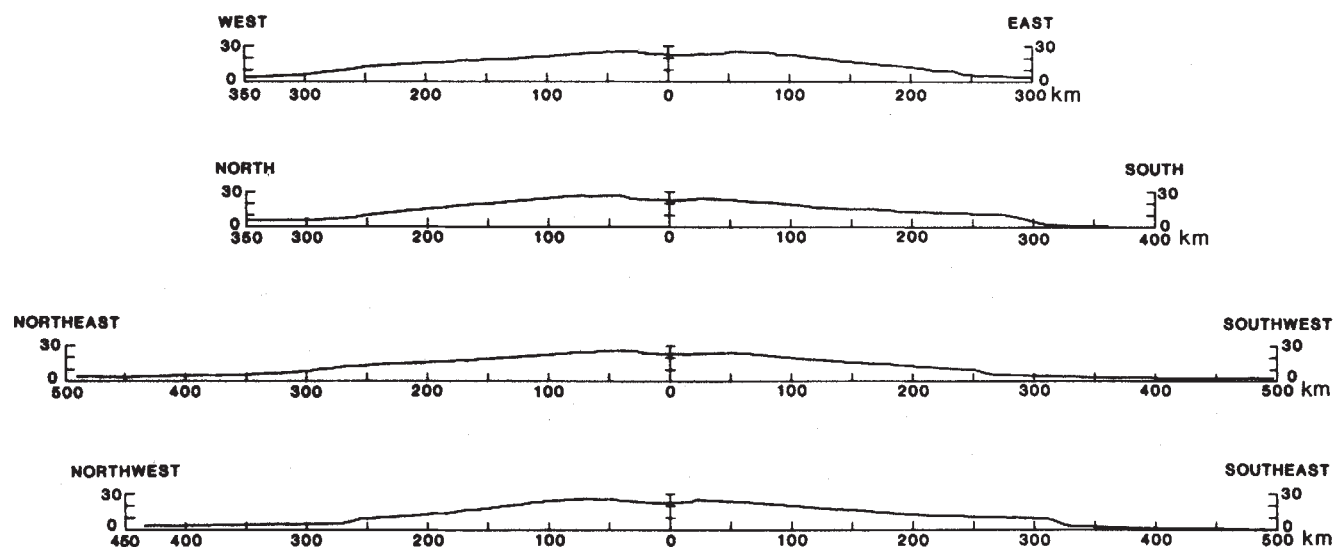


Fig. 3 Profiles measured from the topographic map (Fig. 1) of Olympus Mons. All are drawn so that they pass through the approximate centre of the summit caldera at 18.4° N lat. and 133.45° W long.

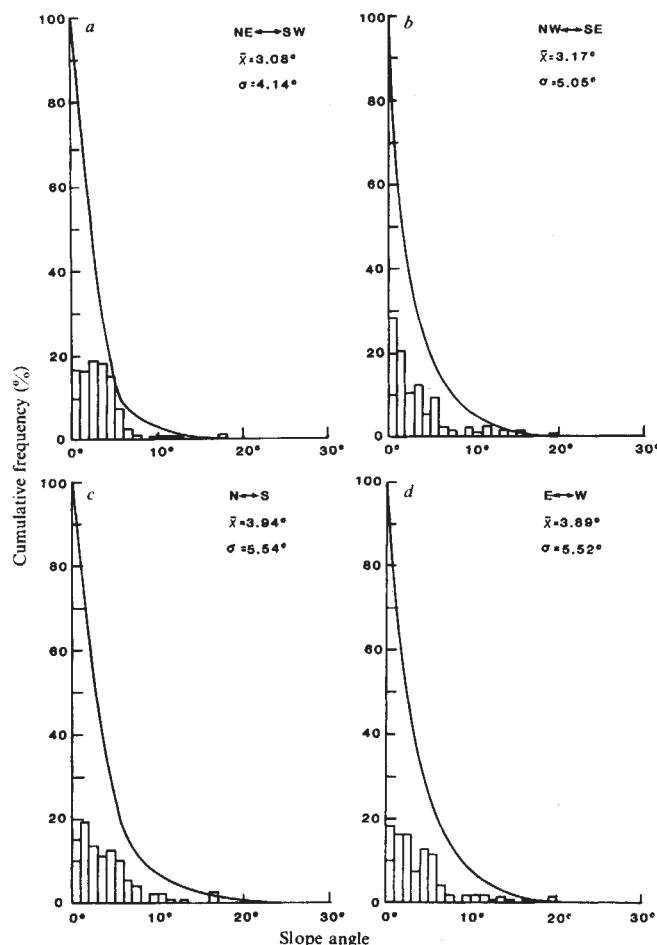


Fig. 4 Slope-frequency distributions of Olympus Mons flanks. Bars represent percentage of samples, by 1° increments of slope angle. Curved lines indicate cumulative frequency of slope angle. The quantity $\bar{x}$ is the mean absolute slope and σ is the algebraic standard deviation of the slope. *a-d*, Slopes measured along NE-SW, NW-SE, N-S and E-W directions respectively. The slope base is 5 km. These profiles, which were derived using a method of terrain analysis employing slope-frequency distributions^{23,24}, show the mean absolute slopes measured along the NE-SW, NW-SE, N-S and E-W directions, to be 3.08°, 3.17°, 3.94° and 3.89°, respectively. The algebraic standard slopes are 4.14°, 5.05°, 5.54° and 5.52°. Although the flank surface appears slightly smoother along its NE-SW direction, the surface of Olympus Mons shield is generally rough.

above the plains. Beyond the scarp, curvilinear troughs and ridges form a grooved terrain, the aureole deposits, which surround Olympus Mons. These deposits are some of the most puzzling features of the Olympus Mons region^{1,21}.

Figure 3 shows profiles measured from the map along the east-west, north-south, north-east-south-west and south-east-north-west directions. Slopes are divided, by elevation, into three groups. Within the 2-7-km interval, slopes of 2°-3° prevail; slopes of 8°-24°, between 7 and 16 km, are steeper mainly because there are several steep scarps within this elevation range; upper slopes, from 13 km to the outside rim of the caldera at 24 km (refs 2,22), measure 2.5°-6.5°.

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1. Carr, M. H., Greeley, R., Blasius, K. R., Guest, J. E. & Murray, J. B. *J. geophys. Res.* **82**, 3985-4015 (1977).
2. Wu, S. S. C., Garcia, P. A., Jordan, R. & Schafer, F. J. *3rd int. Colloq. Mars*, LPI Contr. 441, 287-289 (1981).
3. Wu, S. S. C., Elalass, A. A., Jordan, R. & Schafer, F. J. *Planet. Space Sci.* **30**, 45-55 (1982).
4. Batson, R. M., Hall, D. G. & Edwards, K. *NASA TM-80339*, 415 (1979).
5. Arthur, D. W. G. *Photogram. Rec.* **8**, 617-630 (1976).

6. Blasius, K. R. *J. geophys. Res.* **78**, 4411-4423 (1973).
7. Blasius, K. R., Roberts, W. J., Cutts, J. A., Duxbury, T. C. & Glackin, D. L. *10th A. Div. planet. Sci./Am. Ass. Adv. Sci. Meet.*, Pasadena, 4 (1978).
8. Blasius, K. R. & Cutts, J. A. *Icarus* **45**, 87-112 (1981).
9. Davies, M. E. *Icarus* **21**, 230-235 (1974).
10. Davies, M. E. & Arthur, D. W. G. *J. geophys. Res.* **78**, 4355-4394 (1973).
11. Hord, C. W., Barth, C. A. & Stewart, A. I. *Icarus* **17**, 443-456 (1972).
12. Wu, S. S. C., Schafer, F. J., Nakata, G. M., Jordan, R. & Blasius, K. R. *J. geophys. Res.* **78**, 4405-4410 (1973).
13. Wu, S. S. C. *Int. Rev. Ann. Geophys. Cent. Nat. Rech. Scient. AGEPA* 7-37-(1)-1-259, 147-160 (1981).
14. *Topographic Map of Mars* US Geological Survey, 1-961, (1976).
15. Wu, S. S. C. *US geol. Surv. Interagency Rep.* 63 (1975).
16. Wu, S. S. C. *Icarus* **33**, 417-442 (1978).
17. Carr, M. H. *J. geophys. Res.* **78**, 4049-4062 (1973).
18. Plescia, J. B. & Saunders, R. S. *NASA TM-80339*, 241-243 (1979).
19. Carr, M. H. *Scient. Am.* **234**, 32-43 (1975).
20. Morris, E. C. *3rd Int. Colloq. Mars*, LPI Contr. 441, 161-162 (1981).
21. Lopes, R. M. C. & Guest, J. E. *NASA TM-81776*, 176-178 (1976).
22. Wu, S. S. C., Garcia, P. A., Jordan, R. & Schafer, F. J. *NASA TM-84211*, 141-143 (1981).
23. Pike, R. J. & Rozema, W. J. *Ann. Ass. Am. Geogr.* **65**, 499-516 (1975).
24. Rowan, L. C., McCauley, J. F. & Holm E. A. *US geol. Surv. Prof. Pap.* 599-G, 32 (1971).
25. Beatty, J. K. *Sky Telescope* 420-422 (1982).
26. Hiller, K. H., Janle, P., Neukum, G. P. O., Guest, J. E. & Lopes, R. M. C. *J. geophys. Res.* **87**, 9905-9915 (1982).

Can X-ray diffraction distinguish between protium and deuterium atoms?

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Isotopes are not normally considered to be distinguishable by X-ray crystal structure analysis. There is, of course, no problem as far as neutron diffraction is concerned, as neutron scattering amplitudes vary markedly and irregularly from one nuclide to another¹. In particular, the neutron scattering amplitudes of protium and deuterium have opposite signs so that these two isotopes are especially easily identified. Indeed, in a key experiment nearly 20 yr ago, this difference was used together with the anomalous neutron scattering of ⁶Li to establish the absolute configuration of an enzymatically formed α -monodeuterioglucic acid². We discuss here the possibility of distinguishing deuterium from protium by X-ray diffraction on the basis of the difference in the vibrational behaviour of the two isotopic species.

Least-squares analysis of sufficiently accurate and extensive diffraction data provides information not only about the atomic positions but also about the vibrational behaviour. This information is usually expressed as the components of a tensor U_i for each atom, whereby the product $I^T U_i I$ is the mean-square vibrational amplitude of the atom i about its equilibrium position in the direction of the unit vector I (ref. 3). For light X-ray scatterers, such as hydrogen atoms, the tensors U_i are usually contracted into scalars U_i (isotropic vibrational parameters) which represent the spherically-averaged motion.

Examination of the results of recent low-temperature X-ray crystal structure studies indicates that isotropic U values (mean-square vibrational amplitudes) of hydrogen atoms in small to medium-sized organic molecules are typically in the range $150-300 \times 10^{-4} \text{ \AA}^2$ at temperatures around 100 K (refs 4-10). They are usually larger than equivalent isotropic U values of the heavier atoms, partly because of the effect of internal molecular motions and partly because the hydrogen atoms tend to be situated on the outside of the molecules and hence further from the molecular rigid-body librational axes. The corresponding reported standard deviations of U_H (as derived by least-squares analysis) are typically $\sim 30-40 \times 10^{-4} \text{ \AA}^2$ for reasonably good quality 100 K measurements and are perhaps as low as, say, $15-20 \times 10^{-4} \text{ \AA}^2$ for data sets of the highest quality available.

This is about the precision level that would be required to distinguish isotropic U of protium from that of deuterium at the 2σ (98% probability) level. For example, from tables of

Table 1 Isotropic U values ($\times 10^4$) for hydrogen atoms obtained by least-square analysis from data sets I, II and III with different weighting schemes

Data set	$w = 1$		$w = 1/\sigma^2(F_0)$		$w = 1$	
	U ($\text{\AA}^2$)	$d(X-H)$ ($\text{\AA}$)	U ($\text{\AA}^2$)	$d(X-H)$ ($\text{\AA}$)	U ($\text{\AA}^2$)	$d(X-H)$ ($\text{\AA}$)
H(21) I	98 (30)	0.996 (13)				
	105 (24)	0.994 (11)	95 (20)	0.977 (9)	120 (27)	1.09
	84 (25)	0.991 (12)	72 (20)	0.959 (9)	105 (28)	1.09
H(31)	188 (35)	0.985 (15)				
	193 (29)	1.004 (12)	194 (24)	0.940 (10)	224 (34)	1.09
	190 (32)	1.009 (12)	176 (25)	0.936 (11)	221 (36)	1.09
H(32)	224 (38)	0.999 (15)				
	239 (32)	1.022 (12)	175 (23)	0.944 (10)	264 (36)	1.09
	191 (33)	1.030 (12)	141 (24)	0.951 (10)	212 (36)	1.09
H(41)	237 (39)	0.976 (16)				
	242 (32)	0.961 (13)	313 (30)	1.003 (12)	361 (42)	1.05
	267 (37)	0.966 (14)	291 (31)	1.029 (12)	383 (47)	1.05
H(42)	344 (47)	0.929 (18)				
	315 (37)	0.908 (15)	394 (33)	0.808 (13)	443 (49)	1.05
	303 (40)	0.919 (16)	394 (35)	0.827 (14)	408 (49)	1.05
H(43)	285 (43)	0.910 (16)				
	292 (36)	0.859 (14)	356 (32)	0.940 (12)	497 (51)	1.05
	334 (42)	0.882 (15)	362 (35)	0.983 (13)	495 (54)	1.05
H(44)	304 (44)	0.920 (17)				
	225 (32)	0.915 (13)	224 (26)	0.944 (11)	379 (44)	1.05
	266 (38)	0.922 (14)	273 (30)	0.982 (12)	409 (49)	1.05

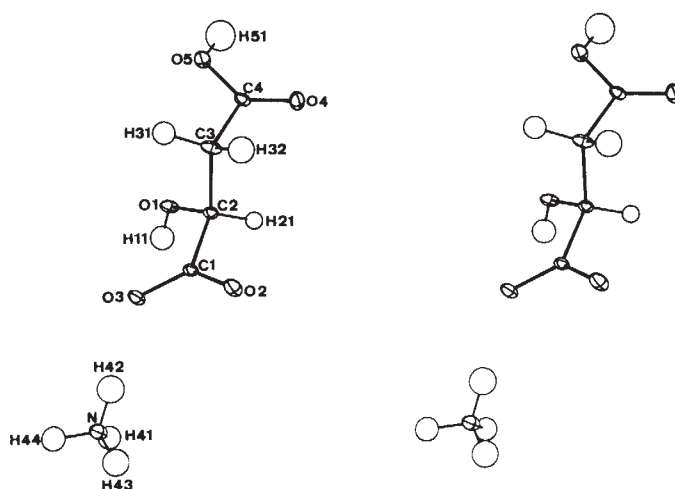
Bond distances obtained from the same calculations and involving the hydrogen atoms are also given. Estimated standard deviations are in parentheses.

molecular vibrational frequencies for propane and 2,2-D₂-propane¹¹, the equivalent isotropic U_H and U_D can be estimated to be $119 \times 10^{-4} \text{ \AA}^2$ for the CH₂ group and $84 \times 10^{-4} \text{ \AA}^2$ for the CD₂ group at 100 K. The difference of $35 \times 10^{-4} \text{ \AA}^2$ should be detectable by X-ray diffraction even if the presence of various systematic errors in this method could raise questions about the absolute level of the U_H values obtained.

We decided to try to determine by X-ray analysis the relative configuration of the two stereogenic centres in deuterium-labelled malic acid obtained by enzymatic addition of D₂O to fumaric acid. Although the answer to this stereochemical question was given more than 20 yr ago^{12,13}, we took the problem up again for methodological reasons, not because of any doubts about the earlier conclusion. Meanwhile an independent confirmation has been provided by a neutron diffraction analysis of the crystalline (+) (*R*)- α -phenylethyl ammonium salt of 2*S*-3-D-malate¹⁴ prepared enzymatically from fumaric acid in a similar way as in the present study. Malic acid itself does not seem to yield suitable well-formed crystals, and we finally settled on the mono-ammonium salt¹⁵ as the best candidate for an accurate low-temperature X-ray diffraction study, a choice that in retrospect may not have been optimal for reasons discussed below.

First, to test whether standard deviations of the structural parameters from such an experiment were reproducible, we measured two independent low-temperature data sets for the undeuterated compound: different crystal specimens, different diffractometers, different low-temperature devices, slightly different temperatures (~ 95 K), and different limiting reflection spheres. We refer to these as data sets I (extending to $\sin \theta/\lambda = 0.90 \text{ \AA}^{-1}$ at 93 K) and II (extending to $\sin \theta/\lambda = 1.19 \text{ \AA}^{-1}$ at 95 K).

The results obtained with these two data sets do not differ in any important respect. As far as the heavy atoms are concerned, atomic positions agree within a few $10^{-4} \text{ \AA}$ and vibration parameters within a few $10^{-4} \text{ \AA}^2$. The hydrogen positions and isotropic U values are sensitive to the scattering factor and weighting system used in the least-squares analyses. Indeed, as Table 1 shows, the variations in the U_H values produced by changing the weighting system within data set II are as large as, or sometimes even larger than, the differences between values obtained from the two data sets using the same (unit) weights. For atom numbering see Fig. 1. The hydroxyl and carboxyl hydrogens, which are engaged in strong hydrogen bonds, are less well defined than the others and have much larger U_H values in both analyses. The ammonium hydrogens, which are also

**Fig. 1** Stereo view of molecule, showing atomic numbering. Vibrational ellipsoids are based on data set II and are drawn at the 50% probability level²¹.

engaged in hydrogen bonds, have larger U_H values, presumably because of rigid-body librational motion of the ammonium group. For the three hydrogen atoms attached to carbon, there is nearly perfect agreement between the U_H values obtained from the two data sets using the same (unit) weights (see Table 1).

This comparison encouraged us to measure diffraction data for a crystal of the mono-ammonium salt of a malic acid sample prepared by enzymatic addition of D₂O to fumaric acid by means of a fumarase preparation from pig heart (Boehringer, Mannheim). Our target was the determination of the relative configuration of the two stereogenic centres, the traditional one at C(2) bearing the hydroxyl group and the new one resulting from isotopic substitution at C(3).

Considerable pains were taken to ensure that the diffraction data for the deuterated crystal (data set III) were measured as closely as possible in the same conditions as data set II for the undeuterated compound. Indeed, the two sets of diffraction measurements including unit-cell dimensions and their temperature dependence are so similar that they could easily be taken for duplicate sets of measurements for the same compound. A parallel, almost perfect correspondence holds for the positional and vibrational parameters of the heavy atoms derived from the two data sets with matching weights (Table 2). In particular, the U_{ij} components of the heavy-atom vibration tensors agree to within a few $10^{-4} \text{ \AA}^2$ units. However, some of the differences, although small, are systematic. First, the b axis of the deuterated crystal is measurably ($\sim 0.01 \text{ \AA}$) longer than that of the undeuterated crystal. This is the direction with the largest coefficient of thermal expansion and is perpendicular to the well-defined layers of molecules that constitute the crystal structure. Table 2 shows that a corresponding increase in the mean-square vibrational amplitudes U_{22} of the heavy atoms in this direction is just detectable.

The U_H values obtained from data sets II and III show greater variation but they could still also be taken as belonging to the same compound. However, given that one of the two methene atoms is protium and one deuterium, the heavier isotope can be identified as H(32) with high probability. On going from data set II to data set III this atom shows a decrease in U_H amounting to $30\text{--}50 \times 10^{-4} \text{ \AA}^2$, depending on the weighting system, whereas its partner H(31) shows nearly constant behaviour. As far as the other hydrogen atoms in this comparison are concerned, some show a slight decrease, some an increase, and some erratic behaviour. Given that the configuration at C(2) is *S*, the one at C(3) is then assigned as *R*, in agreement with previous conclusions^{12–14}.

Unfortunately, the standard deviations of the vibration parameters are $\sim 50\%$ larger than we had hoped to achieve. One

Table 2 Positional parameters (for C, N and O atoms $\times 10^5$, for H atoms $\times 10^4$) and vibrational parameters (all $\times 10^4$) for mono-ammonium 2S-malate (upper value) and 2S-3-D-malate (lower value) at 95 K

		x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N II		92,965 (6)*	86,828 (8)	3,737 (5)	84 (1)	176 (2)	90 (1)	-2 (1)	4 (1)	-5 (1)
N III		92,932 (7)	86,810 (8)	3,736 (5)	85 (1)	175 (2)	91 (1)	-3 (1)	4 (1)	-6 (2)
C(1)		54,602 (6)	85,629 (7)	25,965 (5)	50 (1)	110 (2)	90 (1)	-3 (1)	1 (1)	-6 (1)
		54,590 (7)	85,630 (8)	25,977 (5)	51 (1)	111 (2)	89 (1)	-3 (1)	0 (1)	-5 (1)
C(2)		35,128 (6)	90,278 (7)	26,797 (5)	52 (1)	115 (2)	94 (1)	0 (1)	1 (1)	-2 (1)
		35,118 (7)	90,292 (8)	26,811 (5)	52 (1)	114 (2)	94 (2)	0 (1)	1 (1)	-2 (2)
C(3)		25,395 (6)	77,788 (8)	35,102 (6)	59 (1)	164 (2)	120 (2)	10 (1)	11 (1)	41 (2)
		25,401 (7)	77,835 (9)	35,151 (6)	60 (1)	165 (2)	120 (2)	11 (2)	11 (1)	40 (2)
C(4)		7,209 (6)	84,138 (8)	38,049 (5)	59 (1)	161 (2)	79 (1)	-3 (1)	3 (1)	5 (1)
		7,211 (7)	84,169 (9)	38,085 (5)	59 (1)	161 (2)	78 (1)	-4 (1)	3 (1)	5 (2)
O(1)		26,941 (5)	90,245 (6)	14,768 (4)	66 (1)	177 (2)	96 (1)	-3 (1)	-10 (1)	34 (1)
		26,911 (6)	90,215 (7)	14,784 (4)	66 (1)	177 (2)	96 (1)	-4 (1)	-11 (1)	33 (1)
O(2)		63,384 (5)	86,954 (7)	36,098 (4)	63 (1)	255 (2)	101 (1)	16 (1)	-17 (1)	-45 (1)
		63,382 (6)	86,986 (8)	36,106 (5)	63 (1)	258 (2)	102 (1)	17 (1)	-17 (1)	-46 (2)
O(3)		60,568 (5)	80,966 (6)	15,597 (4)	70 (1)	180 (2)	88 (1)	10 (1)	9 (1)	-17 (1)
		60,546 (6)	80,933 (7)	15,609 (4)	70 (1)	180 (2)	88 (1)	10 (1)	9 (1)	-18 (1)
O(4)		4,532 (6)	96,146 (7)	44,864 (5)	103 (1)	219 (2)	156 (2)	0 (1)	-2 (1)	-75 (2)
		4,519 (7)	96,141 (8)	44,917 (6)	103 (1)	221 (2)	156 (2)	0 (2)	-2 (1)	-76 (2)
O(5)		-5,342 (5)	75,681 (7)	32,471 (5)	62 (1)	207 (2)	142 (1)	-5 (1)	-8 (1)	-51 (1)
		-5,334 (6)	75,728 (8)	32,479 (5)	63 (1)	210 (2)	141 (2)	-5 (1)	-8 (1)	-51 (1)
		x	y	z	U		x	y	z	U
H(11)		3,404	9,584	833	443 (48)	H(42)	8,170	8,377	892	443 (49)
		3,392	9,555	817	495 (54)		8,158	8,362	878	408 (49)
H(21)		3,375	10,253	3,113	120 (27)	H(43)	10,403	8,852	948	497 (51)
		3,369	10,247	3,123	105 (28)		10,413	8,829	938	495 (54)
H(31)		2,423	6,594	3,014	224 (34)	H(44)	9,662	7,744	-264	379 (44)
		2,400	6,595	3,029	221 (36)		9,676	7,760	-274	409 (49)
H(32)		3,204	7,680	4,418	264 (36)	H(51)	-1,702	8,046	3,374	517 (53)
		3,210	7,662	4,420	212 (36)		-1,714	8,020	3,375	441 (51)
H(41)		9,031	9,731	-188	361 (42)					
		9,041	9,714	-206	383 (46)					

* Standard deviations are in parentheses.

reason for this is the markedly anisotropic character of the molecular translational vibration tensor T , due to the strongly layered nature of the crystal structure. For a given atom type, standard deviations of vibrational parameters are closely linearly dependent on the vibrational parameters themselves. Thus for the carbon atoms from data set II (unit weights, Table 2) $\sigma(U_{ii}) = 8.9 + 0.00908 U_{ii}$ (units of $10^{-5} \text{ \AA}^2$) and for the hydrogen atoms (unit weights, Table 1) $\sigma(U) = 17.2 + 0.00630 U$ (units of $10^{-4} \text{ \AA}^2$).

A general reduction of the U_H values would therefore have been very helpful in reducing $\sigma(U_H)$ towards the desired level. This is one reason for thinking that mono-ammonium malate, with its strongly layered crystal structure, may not have been an ideal choice for our experiment. It is also unfortunate that the methene carbon C(3) is the one that shows the largest vibrational motion, a motion that is unavoidably transmitted to the hydrogens it carries. Nevertheless, our experiment suffices to show that with present measurement techniques, the distinction by X-ray diffraction between protium and deuterium atoms in a medium-sized organic molecule is just possible.

Even with large and accurately measured data sets, the X-ray method cannot be expected to match neutron diffraction in its ability to distinguish between protium and deuterium. However, the much wider availability of the X-ray method calls for a critical examination of its potentialities in this direction. We therefore draw attention to several points that will have to be considered by anyone hoping to improve on our work.

First, the crystal chosen for study should have smaller, less anisotropic, vibrational parameters than the one we chose. A slight gain could have been achieved with our crystals by lowering the temperature, but probably not enough to make a significant improvement.

The use of a noncentrosymmetrical, chiral crystal for an experiment of this kind can hardly be avoided as the isotopically labelled, enantiomerically-pure compound must crystallize in a chiral arrangement. At most, crystals of such a compound could be pseudo-centrosymmetric. However, there are well known difficulties associated with the refinement of nearly centrosymmetric structures in noncentrosymmetric space groups¹⁶. From

a symmetry breakdown involving merely a difference between U_H values of two hydrogen atoms, the chance of a successful resolution seems to be remote.

The weighting problem also merits more careful attention than we have devoted to it. The problem with 'normal' weights (inversely proportional to variances) is that too large a weight is assigned to the weak, low-order reflections, which are just the ones most affected by the insufficiency of the free-atom formalism. The contamination of the resulting atomic parameters by the deformation density is especially severe for the hydrogen atoms, leading to the characteristic displacement of these atoms towards their bonded partners and also to less easily recognizable errors in the vibration parameters. These errors in the hydrogen parameters reduce the value of the weighted residual $\Sigma w_i(\Delta F_i)^2$ and hence of the standard deviations as estimated from the least-squares analysis.

Refinements using only high-order reflections give positional and vibrational parameters that differ perceptibly from those obtained from full-data refinements¹⁷. Provided that the high-order data are sufficiently extensive and accurate, the so-derived parameters are less contaminated by the valency electron density and hence, in general, closer to the true values (that is, those obtainable from a neutron-diffraction experiment). High-order refinements on data sets II and III (HO, Table 3) led to heavy-atom parameters with estimated standard deviations less than half of those derived for the full-data refinements and listed in Table 2. The C(2)-H(21) distance refined to 1.08(2) and 1.10(3) Å respectively, that is, virtually to the correct value. The other C-H, N-H, and O-H bond distances still showed deviations (mostly shortenings) of 0.03-0.10 Å. Thus, the high-order reflections contain considerably more signal from H(21) with its appreciably lower U_H than from the other hydrogen atoms. In fact, as Table 1 shows, there is an evident correlation between apparent C-H bond length and U_H : the smaller the C-H distance, the smaller the U_H . This introduces an additional element of uncertainty into the determination of the latter quantities. One possibility is to constrain the C-H, N-H and O-H bond distances to their correct values. This leads to U_H values that are larger, have larger standard deviations, but are more uniform

Table 3 Experimental details for three sets of X-ray diffraction measurements on mono-ammonium 2S-malate (data sets I, II) and mono-ammonium 2S-3-D-malate (data set III)

Data set	I	II	II (HO)	III	III (HO)
Temperature (K)	93	95	95	95	95
sin θ/λ range ($\text{\AA}^{-1}$)	0–0.9	0–1.19	0.7–1.19	0–1.19	0.7–1.19
No. of observed reflections	2,280	5,097	3,996	5,106	4,006
No. of reflections with $I > 3\sigma(I)$	1,882	3,977	2,929	3,888	2,829
No. of variables	128	128	128	128	128
Weight	1	1	$1/\sigma^2(F_0)$	1	$1/\sigma^2(F_0)$
$R(F)$	0.021	0.023	0.017	0.023	0.015
$R_w(F)$	0.024	0.029	0.017	0.032	0.015

between the two data sets II and III (except, of course, for H(32), which shows a drop of $50 \times 10^{-4} \text{\AA}^2$). In retrospect, given that the hydrogen atoms H(31) and H(32) do not scatter significantly at high angles, our experimental effort might have been better spent by making two duplicate sets of measurements out to a lower sin θ/λ limit, say, $0.9 \text{\AA}^{-1}$.

Another question concerns the possibility of measuring the components of the anisotropic tensor U_H instead of just the contracted scalar U_H , for the vibrational amplitudes of the hydrogen atoms can be expected to be far from isotropic. The difficulties are formidable, and no attempt to accomplish this from X-ray diffraction data has been convincing.

Finally, there is the question of whether anything might be gained by expressing the static deformation density in parametric form, and refining the additional parameters together with the usual positional and vibrational parameters by the least-squares method. If sufficient data were available, the decrease in the weighted residual could more than compensate for the increase in the number of parameters, leading to a lowering in the standard deviations of the positional and vibrational parameters.

The X-ray measurements were carried out on two different Enraf-Nonius CAD4 diffractometers each equipped with a graphite monochromator (MoK α radiation, $\lambda = 0.7107 \text{\AA}$) and a modified Enraf-Nonius gas-stream low-temperature device. Temperature fluctuations monitored by a thermocouple for data set I and by a PT100 Ω resistor for data sets II and III were less than $\pm 0.5 \text{ K}$ during the experiments.

The two compounds were recrystallized by slow evaporation of an aqueous solution at about 293 K. Different crystal specimens were used for data sets I and II (space group $P2_12_12_1$). Unit-cell dimensions at 95 K were obtained by least-squares refinement of setting angles for 24 reflections with 2θ values in the range 96° to 105° : data set II, $a = 7.6386(7)$, $b = 8.0254(7)$, $c = 10.5873(8) \text{\AA}$; data set III, $a = 7.6366(6)$, $b = 8.0351(8)$, $c = 10.5840(8) \text{\AA}$. Integrated relative intensities were obtained by ω , 2θ scans.

The room-temperature coordinates¹⁵ served as starting point for our least-squares refinements (X-ray-system¹⁸). Final positional and vibrational parameters, listed in Table 2, are based on full-matrix least-squares analyses using scattering factors for neutral atoms^{19,20} and including an isotropic extinction correction. Additional experimental details for the three data sets are summarized in Table 3.

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- Bacon, G. E. *Applications of Neutron Diffraction in Chemistry* (Pergamon, Oxford, 1963).
- Johnson, C. K., Gabe, E. J., Taylor, M. R. & Rose, I. A. *J. Am. chem. Soc.* **87**, 1802–1804 (1965).
- Dunitz, J. D. *X-Ray Analysis and the Structure of Organic Molecules*, Ch. 1 (Cornell University Press, Ithaca, 1979).
- Ottersen, T. & Hope, H. *Acta crystallogr.* **B35**, 373–378 (1979).
- Stevens, E. D. & Coppens, P. *Acta crystallogr.* **B36**, 1864–1876 (1980).
- Ottersen, T., Almlöf, J. & Hope, H. *Acta crystallogr.* **B36**, 1147–1154 (1980).
- Dunitz, J. D., Schweizer, W. B. & Seiler, P. *Helv. chim. Acta* **66**, 134–137 (1983).
- Hope, H. & Ottersen, T. *Acta crystallogr.* **B34**, 3623–3626 (1978).
- Stevens, E. D. *Acta crystallogr.* **B34**, 544–551 (1978).
- Savariault, J.-M. & Lehmann, M. S. *J. Am. chem. Soc.* **102**, 1298–1303 (1980).

- Shimanouchi, T. *Tables of Molecular Vibrational Frequencies, Consolidated Vol. I* (NSRDS-NBS 39, US Department of Commerce, 1972).
- Gawron, O. & Fondy, T. P. *J. Am. chem. Soc.* **81**, 6333–6334 (1959).
- Anet, F. A. L. *J. Am. chem. Soc.* **82**, 994–995 (1960).
- Bau, R. *et al. Biochem. biophys. Res. Commun.* **115**, 1048–1052 (1983).
- Versichel, W., van de Mieroop, W. & Lenstra, A. T. H. *Acta crystallogr.* **B34**, 2643–2645 (1978).
- Ermer, O. & Dunitz, J. D. *Acta crystallogr.* **A26**, 163 (1970).
- Seiler, P., Schweizer, W. B. & Dunitz, J. D. *Acta crystallogr.* (in the press).
- Stewart, J. M., Kruger, G. J., Ammon, H. L., Dickinson, C. & Hall, S. R. *The X-ray System, Version of June 1972* (Tech. Rep. TR192, University of Maryland, 1972).
- Cromer, D. T. & Mann, J. B. *Acta crystallogr.* **A24**, 321–324 (1968).
- Stewart, R. F., Davidson, E. R. & Simpson, W. T. *J. chem. Phys.* **42**, 3175–3187 (1965).
- Johnson, C. K. *Ortep Rep. ORNL-3794* (Oak Ridge National Laboratory, Tennessee, 1965).

Microgranitoid enclaves in granites—globules of hybrid magma quenched in a plutonic environment

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Microgranitoid enclaves (autoliths, cognate xenoliths, mafic inclusions) are common in high-level granitoid plutons. They have relatively fine-grained igneous (microgranitoid) microstructures¹, and many show evidence of having flowed in a magmatic condition². These features counter interpretations that the enclaves represent transformed solid fragments of diverse wall rocks^{3–5} or restite^{6–11}. An alternative interpretation is that the enclaves represent globules of mafic magma that have mingled ('commingled') and quenched in the granitoid host magma^{12–16}. A detailed review of the literature, occurrence, morphology and composition of microgranitoid enclaves strongly supports this view, but indicates that the enclave magmas range in composition from mafic to felsic, and that magma-mixing may be involved in their formation¹⁷. I summarize here the main features of microgranitoid enclaves and briefly outline the possible processes leading to their incorporation as magma globules in the host granitoid.

The microgranitoid enclaves occur in both I-type and S-type high-level granitoid plutons^{17,18}. Their abundance is not related to pluton contacts nor the number of hornfels xenoliths², and they are typically much more abundant than hornfels or other xenoliths¹⁷. Their distribution may be locally uniform, but is commonly irregular, and they may be concentrated in swarms². They are generally unrepresented among the exposed country rocks⁶.

The microgranitoid enclaves typically have rounded to ovoid shapes^{1,2}, though sub-angular and, more rarely, angular enclaves may be present². Some have cusped margins, with lobes convex towards the host granitoid. Elongate enclaves are markedly ellipsoidal to lenticular, and are aligned in areas of the granitoid that show a prominent primary flow foliation², especially near the margins of the pluton⁷. Their alignment and degree of elongation correlate with the intensity of the flow foliation in the host granitoid^{2,17}. Neither enclave nor granitoid show microstructural evidence of crystal plasticity or recrystallization, so that the extension appears to have been accomplished by relative movement of crystals and melt; that is, when they were in a magmatic condition^{17,19}.

The enclaves generally have uniform, random microstructures, though some have finer-grained margins. Some have flow alignment of plagioclase or hornblende crystals, which may be parallel to the enclave margins and may be deflected around phenocrysts. The enclave microstructures are typical of fine- to medium-grained igneous rocks, consisting commonly of euhedral phenocrysts of zoned plagioclase, with or without quartz, biotite, hornblende (in I-types) or orthopyroxene $\pm$ cordierite (in

S-types), set in a groundmass rich in biotite and euhedral laths of zoned plagioclase, commonly with interstitial to poikilitic quartz, and/or K-feldspar¹⁷. They typically contain apatite needles, indicative of magma quenching²⁰. They have compositionally and microstructurally similar counterparts in calc-alkaline volcanic rocks^{13,14,21,22}; many of these contain interstitial glass and some have skeletal, zoned crystals of hornblende and plagioclase, indicating a magmatic condition^{13,14,22}.

The enclaves are darker and finer-grained than their host granitoids, but although some are mafic¹⁶, most are intermediate to silicic²³. Many compositional varieties may occur together. The mineral assemblages in the enclaves are generally the same as those in the host granitoid, but the proportions and compositions of the minerals are typically different^{2,17}.

Magmatic orthopyroxene + cordierite + plagioclase in the microgranitoid enclaves of the S-type Cowra Granodiorite, New South Wales, Australia¹⁸, indicate crystallization at relatively low confining pressures (100–200 MPa)²⁴. If these enclaves are representative, microgranitoid enclaves appear to crystallize at pluton emplacement levels, which agrees with the observation that they are restricted to relatively high-level plutons²⁵.

The inference that the enclaves were globules of magma at the time that the flow occurred can explain the typical rounded to cusped shapes of the enclaves. The rounded shapes appear to be primary, rather than due to rounding of angular, solid fragments, because nearby hornfels xenoliths generally show little or no rounding. The more angular enclaves could be fragments of solid, quenched igneous rocks, but they could also be magma fragments, because magma may break (like pitch) under rapidly applied stress²⁶.

The relatively fine-grained microstructures indicate relatively rapid crystallization; that is, quenching of magma in the plutonic environment. Examples of mafic (mafic to intermediate) magma quenched against felsic magma are common in volcanic^{22,26–28}, subvolcanic^{12,26,29–31} and plutonic^{16,19,29,32–37} environments, as discussed in detail elsewhere¹⁷. The process involves magma mingling, whereby rounded to cusped globules and streaks of the more mafic magma become dispersed through the felsic magma. The more mafic magma crystallizes more rapidly (forming finer-grained aggregates) than the felsic magma, because it is more undercooled than the felsic magma²², once thermal equilibrium between the two has been established. Mafic enclave magmas would be much more strongly undercooled than their granitoid host magmas²². However, even where the enclave and granitoid magmas are fairly similar in composition, the more mafic enclave magma can crystallize more rapidly, because small differences in the degree of undercooling can give rise to marked increases in nucleation rate and marked decreases in growth rate¹⁷.

The origin of the magma that produced the enclaves is a matter for speculation, as examples of magma mingling show only that magmas can intimately coexist, not how they originated¹¹. Though felsic and more mafic calc-alkaline magmas are chemically miscible^{37–40}, they can remain separate³⁸, particularly if small volumes of the more mafic magma are enclosed in large volumes of felsic magma and if stirring is minimal^{39,40}, because chilling increases the viscosity of the more mafic globules, as well as promoting rapid crystallization.

Enclave magmas conceivably could be derived from: (1) partly crystallized, quenched margins of the granitoid^{18,41} (if this can account for the evidence of hybridism and the observed compositional range of enclaves in a single pluton), (2) repeated mingling of a variety of more mafic (?) hybrid magmas injected into, or fortuitously encountered by, the granitoid, or (3) a more mafic lower layer in the pluton that is partly mixed with the granitoid magma¹⁷. Careful isotopic studies could conceivably indicate whether the enclave magmas are closely related to the granitoid magma⁴² (for example, either differentiates of it or separate batches of (?) hybrid magma from the same or an isotopically similar source) or whether they involve contributions from unrelated magmas²².

One way of producing the observed compositional variation of the enclaves is by magma mixing, which involves homogenization of the two melts (in contrast to magma mingling). The common presence of plagioclase with resorbed or dendritic cores, K-feldspar megacrysts with oligoclase rims, and rounded quartz xenocrysts with fine-grained mafic rims indicates that many enclave magmas are related in origin to rocks showing mineralogical evidence of hybridism^{17,28,29}, for example, the Skye marscoite, for which magma mixing is commonly postulated^{29,43}. Therefore, the enclave magmas may be products of magma mixing¹⁵, either in the same pluton¹⁷, or at a site removed from the actual magma mingling in the pluton, as commonly appears to be so for clear examples of magma mingling^{29,30}.

Reid *et al.*¹⁶ recently presented clear evidence of the mingling of felsic magma with more mafic magma globules in the Sierra Nevada, California, adding that solution and disintegration of the resulting enclaves contaminated the surrounding granitoid. However, in my experience, microgranitoid enclaves typically show little or no evidence of disintegration, and the surrounding granitoid is generally not obviously modified³⁶. Furthermore, disintegration of enclaves should disseminate finer-grained material than is typical of granitoids. The tendency of enclaves to be more abundant in more mafic granitoids^{8,16} may be due simply to local accumulation of enclaves and precipitated crystals⁴⁴. The common coexistence of enclaves of variable composition¹⁷ and the microstructural evidence of hybridism suggest that the compositional variety of the enclave magmas was produced away from the exposed site of final mingling. Furthermore, reports of 'lamprophyric' dykes with corroded xenocrysts, resembling enclaves in adjacent granitoids^{29,45}, suggest that enclave magmas can exist either independently of, or as a separate layer in, their host granitoid magma bodies.

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- Phillips, J. A. *Q. Jl geol. Soc. Lond.* **36**, 1–21 (1880).
- Pabst, A. *Univ. Calif. Publ. geol. Sci.* **17**, 325–386 (1928).
- Bowen, N. L. *J. Geol.* **30**, 513–570 (1922).
- Nockolds, S. R. *J. Geol.* **41**, 561–589 (1933).
- Grout, F. F. *Bull. geol. Soc. Am.* **48**, 1521–1572 (1937).
- Chappell, B. W. *J. geol. Soc. Aust.* **25**, 267–283 (1978).
- Bateman, P. C., Clark, L. D., Huber, N. K., Moore, J. G. & Rinehart, C. D. *U.S. geol. Surv. Prof. Pap.* **414-D**, (1963).
- White, A. J. R. & Chappell, B. W. *Tectonophysics* **43**, 7–22 (1977).
- Hine, R., Williams, I. S., Chappell, B. W. & White, A. J. R. *J. geol. Soc. Aust.* **25**, 219–234 (1978).
- Griffin, T. J., White, A. J. R. & Chappell, B. W. *J. geol. Soc. Aust.* **25**, 235–247 (1978).
- McBirney, A. R. *J. Volcanol. geotherm. Res.* **7**, 357–371 (1980).
- Walker, G. P. L. & Skelhorn, R. R. *Earth-Sci. Rev.* **2**, 93–109 (1966).
- Eichelberger, J. C. *Nature* **275**, 21–27 (1978).
- Eichelberger, J. C. *Nature* **288**, 446–450 (1980).
- Hibbard, M. J. *Contr. Miner. Petrol.* **76**, 158–170 (1981).
- Reid, J. B., Evans, O. C. & Fates, D. C. *Earth planet. Sci. Lett.* **66**, 243–261 (1983).
- Vernon, R. H. *J. Proc. R. Soc. N.S.W.* **116**, 77–103 (1983).
- Vernon, R. H. & Flood, R. H. in *New England Geology*, 201–210 (University of New England, Armidale, 1982).
- Vernon, R. H., Etheridge, M. A. & Wall, V. J. *Abstr. geol. Soc. Aust.* **9**, 185 (1983).
- Wyllie, P. J., Cox, K. G. & Biggar, G. M. *J. Petrol.* **3**, 238–243 (1962).
- Wilkinson, J. F. G., Vernon, R. H. & Shaw, S. E. *J. Petrol.* **5**, 461–488 (1964).
- Zeck, H. P. *Contr. Miner. Petrol.* **26**, 225–246 (1970).
- Didier, J. *Granites and their Enclaves* (Elsevier, Amsterdam, 1973).
- Clemens, J. D. & Wall, V. J. *Can. Miner.* **19**, 111–131 (1981).
- Fershtater, G. B. & Borodina, N. S. *Int. Geol. Rev.* **19**, 458–468 (1977).
- Blake, D. H., Elwell, R. W. D., Gibson, I. L., Skelhorn, R. R. & Walker, G. P. L. *Q. Jl geol. Soc. Lond.* **121**, 31–50 (1965).
- Sparks, R. S. J., Siquardson, H. & Wilson, L. *Nature* **267**, 315–318 (1977).
- Van Bergen, M. J., Ghezzi, C. & Ricci, C. A. *J. Volcanol. geotherm. Res.* **19**, 1–35 (1983).
- Harker, A. *The Natural History of the Igneous Rocks* (Methuen, London, 1909).
- King, B. C. *Sci. Prog.* **52**, 282–292 (1964).
- Gamble, J. A. *J. Volcanol. geotherm. Res.* **5**, 297–316 (1979).
- Wells, A. K. & Woolridge, S. W. *Proc. geol. Ass.* **42**, 178–215 (1931).
- Blake, D. H. *BMJ J. Aust. Geol. Geophys.* **6**, 95–99 (1981).
- Wiebe, R. A. *Am. J. Sci.* **273**, 130–151 (1973).
- Wiebe, R. A. *J. Geol.* **82**, 74–87 (1974).
- Thomas, H. H. & Smith, W. C. *Q. Jl geol. Soc. Lond.* **88**, 274–296 (1932).
- Taylor, T. R., Vogel, T. A. & Wilbrand, J. T. *J. Geol.* **88**, 433–444 (1980).
- Yoder, H. S. *Am. Miner.* **58**, 153–171 (1973).
- Kouchi, A. & Sunagawa, I. *Sci. Rep. Tohoku Univ. Ser. 3* **15**, 163–175 (1982).
- Kouchi, A. & Sunagawa, I. *Nature* **304**, 527–528 (1983).
- Phillips, G. N., Wall, V. J. & Clemens, J. D. *Can. Miner.* **19**, 47–63 (1981).
- Williams, I. S., Compston, W. & Chappell, B. W. *J. Petrol.* **24**, 76–97 (1983).
- Wager, L. R., Vincent, E. A., Brown, G. M. & Bell, J. D. *Phil. Trans. R. Soc. A* **257**, 273–308 (1965).
- Clemens, J. C., Wall, V. J. & Clarke, B. D. *Abstr. geol. Soc. Aust.* **9**, 178–179 (1983).
- Harker, A. & Marr, J. E. *Q. Jl geol. Soc. Lond.* **47**, 266–328 (1891).

Inverse relationship between Sr isotope diversity and rate of oceanic volcanism has implications for mantle heterogeneity

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Cohen and O'Nions¹ noted that basalts from the Mid-Atlantic Ridge (MAR) display greater diversity of Pb, Nd and Sr isotopes than do basalts from the East Pacific Rise (EPR). They attributed this difference not to greater isotopic heterogeneity beneath the MAR, but rather to more effective mixing (to eradicate heterogeneity) beneath the EPR. Allègre *et al.*² reached the same conclusion on the basis of isotope data including noble gas isotopic data. To test this idea further, I compare here the average spreading rate of nine portions of the mid-ocean ridge system with the measured $^{87}\text{Sr}/^{86}\text{Sr}$ diversity of basalts. This shows that the maximum observed $^{87}\text{Sr}/^{86}\text{Sr}$ diversity is inversely proportional to spreading rate. Furthermore, globally averaged eruption rates for mantle-derived rocks of intra-oceanic island arcs, ocean islands and the mid-ocean ridge system exhibit a negative correlation with $^{87}\text{Sr}/^{86}\text{Sr}$ diversity. These results suggest that the upper mantle is everywhere heterogeneous on a small scale and that the extent of observed heterogeneity is a function of mixing. Independent geophysical and petrological evidence for the existence of steady-state crustal magma chambers beneath fast-spreading ridges and their absence below slow spreading ridges favours this hypothesis because mixing is enhanced by the presence of a magma chamber. Alternatively, mixing during melt production in the mantle, during melt segregation or during ascent could also result in the observed patterns if the degree of mixing is related to eruption rate. Large heterogeneities (mantle plumes?) are required to explain why isotopic variations are well correlated geographically in some places but not in others.

Several workers have suggested that the sub-oceanic mantle source for basalt melts is ubiquitously heterogeneous on a small scale³⁻⁶. A 'plum pudding' mantle of this type can, on melting, produce suites of basalts with variable $^{87}\text{Sr}/^{86}\text{Sr}$. The variability depends partly on the sizes of the isotopically heterogeneous regions compared with the size of the region undergoing batch melting. Batch melting of regions that are large compared with the sizes of isotopically heterogeneous domains will produce a

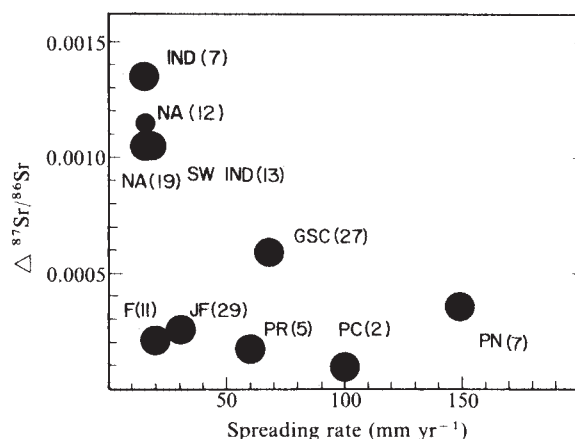


Fig. 1 Plot of $^{87}\text{Sr}/^{86}\text{Sr}$ range (Δ) against spreading rate for the data of Table 1. Numbers in parentheses are the number of samples. IND, Indian Ridge; NA, North Atlantic; SW IND, south-west Indian Ocean Ridge; F, FAMOUS Area; JF, Juan de Fuca Ridge; GSC, Galapagos Spreading Centre; PR, Pacific-Rivera (21°N); PC, Pacific-Cocos and PN, Pacific Nazca Ridge. For available high-precision analyses of glasses and carefully leached samples, there is an inverse correlation between the maximum strontium isotope diversity of MORB and the spreading rate.

homogeneous melt with isotopic characteristics that approach those of the bulk source. On the other hand, batch melting of smaller regions produces melts that selectively sample the heterogeneities. Melting of several such small isolated regions would produce a suite of melts with diverse isotopic characteristics that, as a group, reflect the isotopic diversity of the source rather than its average bulk characteristics.

Likewise, if the size of the melt region is held constant, melts produced by batch melting of plum pudding mantles would be expected to have isotopic characteristics that vary as a function of the extent of partial melting^{4,7,8}. Small degrees of partial melting would selectively sample mantle domains having lower solidus temperature because of enrichments in volatiles, large ionic lithophile (LIL) elements and ^{87}Sr and larger extents of melting would result in dilution of this melt fraction with melt from more refractory LIL-poor mantle domains^{9,10}. Suites of isotopically diverse melts could be produced in this way by lateral, depth or temporal variations in the extent of partial melting.

Isotopically diverse melts that are generated simultaneously in neighbouring regions of a plum pudding mantle by either variable extents of batch melting or variable size of melt region or both, may ascent and erupt without mixing to produce isotopi-

Table 1 Sr-isotopic, spreading rate and eruption rate data

Location	Highest $^{87}\text{Sr}/^{86}\text{Sr}$	Lowest $^{87}\text{Sr}/^{86}\text{Sr}$	Δ $^{87}\text{Sr}/^{86}\text{Sr}$	Data sources	No. of samples	Spreading rate (mm yr ⁻¹)
Indian Ocean	0.70397 ± 9	0.70262 ± 3	0.00135	42	7	10–20
SW Indian Ridge	0.70372 ± 6	0.70267 ± 4	0.00105	17	13	17.2
North Atlantic	0.70357	0.70251	0.00106	15	19*	10–20
North Atlantic	0.70346 ± 4	0.70231 ± 6	0.00115	16	12	10–20
Juan de Fuca Ridge	0.70263 ± 6	0.70237 ± 6	0.00026	14	29	30
Pacific–Nazca Ridge	0.70287 ± 5	0.70251 ± 5	0.00036	1, 43, 44	7	150
Pacific–21° N	0.70264 ± 8	0.70247 ± 6	0.00017	45	5	60
FAMOUS Rift	0.70296 ± 6	0.70276 ± 11	0.00020	45	11	20
Galapagos Spreading Centre	0.70307 ± 3	0.70248 ± 4	0.00059	46	27	65–70
Pacific–Cocos	0.70259 ± 3	0.70250 ± 4	0.00009	4	2	100
						Eruption rate† (km ³ yr ⁻¹)
Mid Ocean Ridges—compilation			0.0012	7	—	22
Ocean Islands—compilation			0.0042	7	—	1.5
Intra-Oceanic Island Arcs—compilation			0.0029	7	—	3.0

* Mean analyses.

† From ref. 18.

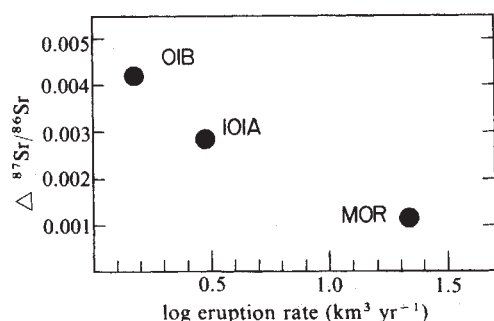


Fig. 2 Plot of $^{87}\text{Sr}/^{86}\text{Sr}$ range (Δ) against global, time-averaged eruption rate for mid-ocean ridges (MOR), ocean islands (OIB) and intra-oceanic island arcs (IOIA). $^{87}\text{Sr}/^{86}\text{Sr}$ isotope compilations are from ref. 7.

cally diverse lava suites. Alternatively, mixing of these diverse batches in the mantle or crust could partly or completely homogenize the diverse melts.

Table 1 is a compilation of published ranges of high-precision $^{87}\text{Sr}/^{86}\text{Sr}$ data for mid-ocean ridge basalts (MORB) with corresponding spreading rates^{11–14}. These are measured values for fresh glasses and carefully leached crystalline basalts. Figure 1 shows that the maximum observed range of $^{87}\text{Sr}/^{86}\text{Sr}$ is inversely correlated with spreading rate for MORB; smaller ranges of variation are possible for smaller geographical subsets or may be artificially created by insufficient sampling. Ideally, of course, this sort of comparison is most meaningful if normalized to equal ridge crest lengths that are sampled with uniform density. Unfortunately, the available data are too fragmentary for this, or for rigorous statistical analysis of the variation along all ridge crest segments. Note that Fig. 1 contains data for ridge crest segments that display coherent geographical patterns of isotope variation (like the northern MAR^{15,16}) as well as slow-spreading ridges that do not (like the south-west Indian Ocean Ridge.¹⁷)

Table 1 also gives $^{87}\text{Sr}/^{86}\text{Sr}$ data compiled by Morris and Hart⁷ for mantle-driven intra-oceanic island arcs, oceanic islands and the mid-ocean ridge system together with globally-integrated average eruption rate estimates of Crisp¹⁸. For these data, average eruption rate is inversely correlated with diversity of $^{87}\text{Sr}/^{86}\text{Sr}$ (Fig. 2). This agrees with the MORB data if the eruption rate at mid-ocean ridges is proportional to spreading rate.

Future additional sampling and analyses will be required to determine whether the patterns of Fig. 1 hold up or are merely artefacts. For future sampling of ridge crests it is important to distinguish between the axes of normal ridge crests and small, isotopically diverse seamounts that may be present near the ridge⁸. In addition, recently discovered petrological effects at ridge transform intersections^{19–21} may be important, so it is crucial to know the locations of transforms and ridge off-sets^{22,23}.

If these general patterns are confirmed, there are several possible explanations for their existence. It is possible, of course, that the mantle sources of slow-spreading ridges are simply more heterogeneous than the sources below fast-spreading ridges. Alternatively, the extent of contamination of basalt magma may be related to spreading rate²⁴. These seem unlikely, however, and I prefer to interpret these patterns in terms of melting of a plum pudding mantle associated with or followed by varying amounts of homogenization caused by mixing. This is reasonable in view of the large amount of independent geophysical and petrological evidence for the spreading rate dependence of steady state crustal magma chambers beneath ridge axes^{25–29}. It is also possible, of course, that mixing takes place in the mantle during melting^{30–32}, segregation^{33–35} or ascent³⁶. Evaluation of this possibility must await better understanding of the thermal structure beneath ridges, their temporal variability and possible feed back and buffering of the thermal and rheologic properties of the mantle by melting and magmatic ascent processes^{37–39}. If our preferred interpretation of the pattern in Figs 1 and 2 is correct, it has important implications for the nature and size of

mantle reservoirs, their convective mixing^{40,41} and their thermal and differentiation histories.

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- Cohen, R. S. & O'Nions, R. K. *J. Petrol.* **23**, 299–324 (1982).
- Allègre, C. J., Dupre, B. & Hamelin, B. *Trans. Am. geophys. Un.* **64**, 324 (1983).
- Zindler, A., Hart, S. R., Frey, F. A. & Jakobsson, S. P. *Earth planet. Sci. Lett.* **45**, 249–262 (1979).
- Zindler, A., Staudigel, H. & Batiza, R. *Earth planet. Sci. Lett.* (in the press).
- Allègre, C. J., Brevart, O., Dupre, B. & Minster, J.-F. *Phil. Trans. R. Soc. A297*, 447–477 (1980).
- Davies, G. F. *Nature* **290**, 208–213 (1981).
- Morris, J. D. & Hart, S. R. *Geochim. cosmochim. Acta* **47**, 2015–2030 (1983).
- Batiza, R. & Vanko, D. *J. geophys. Res.* (in the press).
- Gurney, J. J. & Harte, B. *Phil. Trans. R. Soc. A297*, 273–293 (1980).
- Frey, F. A. & Prinz, M. *Earth planet. Sci. Lett.* **38**, 129–176 (1978).
- Lonsdale, P. *Mar. geophys. Res.* **3**, 251–293 (1977).
- Heizler, J. R., Dickson, G. O., Herron, E. M., Pitman, W. C. & LePichon, X. *J. geophys. Res.* **73**, 2119–2136 (1968).
- Sclater, J. G. *et al. J. geophys. Res.* **81**, 1857–1869 (1976).
- Eaby, J., Clague, D. A. & Delaney, J. R. *J. geophys. Res.* (in the press).
- Schilling, J.-G. *et al. Am. J. Sci.* **283**, 510–586 (1983).
- Dupre, B. & Allègre, C. J. *Nature* **286**, 17–22 (1980).
- LeRoex, A. P. *et al. J. Petrol.* **24**, 267–318 (1983).
- Crisp, J. A. *J. volcanol. Geotherm. Res.* **20**, 177–211 (1984).
- Bender, J. F., Langmuir, C. H. & Hanson, G. N. *J. Petrol.* **25**, 213–254 (1984).
- Perfit, M. R., Fornari, D. J., Malahoff, A. & Embley, R. W. *J. geophys. Res.* **88**, 10,551–10,572 (1983).
- Schilling, J.-G. & Sigurdsson, H. *Nature* **282**, 370–375 (1979).
- Macdonald, K. C. & Fox, P. J. *Nature* **302**, 55–58 (1983).
- Lonsdale, P. *Bull. geol. Soc. Am.* (in the press).
- O'Hara, M. J. & Mathews, R. E. *J. geol. Soc. Lond.* **138**, 237–277 (1981).
- Rosendahl, B. R. *J. geophys. Res.* **81**, 5305–5314 (1976).
- Natland, J. H. *DSDP Init. Rep. Leg 54*, 833–850 (1980).
- Orcutt, J. *et al. Nature* **256**, 475–476 (1975).
- Morel, J. M. & Hekinian, R. *Contr. Miner. Petrol.* **72**, 425–436 (1980).
- Bryan, W. B. & Moore, J. B. *Bull. geol. Soc. Am.* **88**, 556–570 (1977).
- Langmuir, C. H., Bender, J. F., Bence, A. E., Hanson, G. N. & Taylor, S. R. *Earth planet. Sci. Lett.* **36**, 133–156 (1977).
- Hanks, T. C. *J. geophys. Res.* **76**, 537–544 (1971).
- Turcotte, D. L. & Ahern, J. L. *J. geophys. Res.* **83**, 767–772 (1978).
- Bottinga, Y. & Allègre, C. J. *Phil. Trans. R. Soc. A278*, 501–525 (1978).
- Sleep, N. H. *Bull. geol. Soc. Am.* **85**, 1225–1232 (1974).
- Stolper, E., Walker, D., Hager, B. H. & Hays, J. F. *J. geophys. Res.* **86**, 6261–6271 (1981).
- Spera, F. in *Physics of Magmatic Processes* (ed. Hargraves, R. B.) 268–324 (Princeton University Press, 1980).
- Presnall, D. C., Dixon, J. R., O'Donnell, T. H. & Dixon, S. A. *J. Petrol.* **20**, 3–35 (1979).
- Brace, W. F. & Kohlstedt, D. L. *J. geophys. Res.* **86**, 6258–6252 (1980).
- Feigenson, M. D. & Spera, F. *J. Geology* **9**, 531–533 (1981).
- Richter, F. M. & Ribe, N. M. *Earth planet. Sci. Lett.* **43**, 212–222 (1979).
- Richter, F. M., Daly, S. F. & Nataf, H.-C. *Earth planet. Sci. Lett.* **60**, 178–194 (1982).
- Dupre, B. & Allègre, C. J. *Nature* **303**, 142–146 (1983).
- White, W. M. & Hoffman, A. W. *Nature* **296**, 821–825 (1982).
- Cohen, R. S., Evensen, N. M., Hamilton, P. J. & O'Nions, R. K. *Nature* **283**, 149–153 (1980).
- Dupre, B., Lambret, B., Rousseau, D. & Allègre, C. J. *Nature* **294**, 552–554 (1981).
- Verna, S. P. & Schilling, J.-G. *J. geophys. Res.* **87**, 838–10,856 (1982).

Particulate and dissolved vanadium in the North Pacific Ocean

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The distributions of dissolved trace elements in the oceans result from a complex series of biogeochemical processes. Historically, most models describing the cycles of these elements have been formed around high quality data for the distributions of the dissolved elements¹. I report here a detailed vertical profile of particulate vanadium and estimate its particle-associated flux. This flux suggests a surface depletion of dissolved vanadium which is verified by the first detailed determinations of dissolved vanadium in several profiles from the Pacific Ocean.

Particulate matter was collected from the upper 1,500 m of the Panama Basin by a large volume filtration system (LVFS)². The samples were collected in July 1976 and again in March 1979 from the same location, 0° 44'N, 86° 10'W. The chemistry, biology, and vertical flux of material in the 1976 samples have been reported elsewhere³ and a discussion of the 1979 samples is in preparation. Samples for dissolved vanadium analysis were collected from this site and several other locations in the North Pacific Ocean (Table 1).

The concentration of particulate vanadium in the $>53\ \mu\text{m}$ particles is shown in Fig. 1. The basic form of these two vertical profiles is driven by the distribution of total particulate mass³. The strong surface maximum is associated with the primary production of biogenic particulate matter. However, terrigenous

Table 1 Hydrographic data and vanadium concentrations in vertical profiles from the North Pacific Ocean

Depth (m)	Temperature (°C)	Potential salinity	O ₂ ($\mu\text{mol l}^{-1}$)	PO ₄ ($\mu\text{mol l}^{-1}$)	V (nmol l ⁻¹)
MANOP S					
1	26.97	34.676	205	0.08	32.0
50	26.86	34.671	229	0.03	32.6
100	13.47	34.588	12.5	2.21	33.3
150	11.51	34.730	14.3	2.27	34.4
200	10.87	34.720	15.2	2.30	34.9
300	9.936	34.693	24.0	2.39	35.0
350	9.61	34.679	11.6	2.40	35.3
400	9.321	34.664	12.5	2.50	35.3
450	8.97	34.643	7.1	2.61	35.0
497	8.435	34.609	10.0	2.75	34.8
546	7.868	34.575	7.7	2.75	37.1
595	7.47	34.561	7.0	2.97	36.5
693	6.34	34.526	13.2	3.03	36.1
742	5.901	34.527	14.4	2.96	37.4
890	4.985	34.533	29.9	3.00	36.6
988	4.518	34.555	40.0	3.08	36.5
1,185	3.853	34.569	48.4	2.95	36.0
1,280	3.592	34.578	68.0	2.72	35.6
1,478	3.122	34.596	65.3	2.73	36.5
1,675	2.722	34.611	76.3	2.66	34.9
1,872	2.362	34.628	88.2	2.54	36.4
2,070	2.11	34.642	97.2	2.35	35.7
2,267	1.96	34.657	108	2.38	35.9
2,677	1.794	34.666	117	2.37	36.2
2,975	1.698	34.673	127	2.34	36.3
3,472	1.551	34.681	138	2.25	35.3
4,625	1.444	34.693	167	2.14	36.1
4,772	1.442	34.695	175	2.16	36.4
4,904	1.432	34.694	169	2.18	36.6
Galapagos					
10	24.94		218	0.51	33.6
40	20.67		165	0.98	34.0
80	17.87		135		34.7
120			98	1.57	35.3
200	14.52		96	1.64	35.3
250	13.54		74	1.87	35.9
300	12.16		19	2.38	35.9
350	10.66		10	2.60	36.1
400	9.40		11	2.79	35.7
500	7.73		23	2.96	36.6
600	6.96		37	3.00	37.4
800	5.62		54		36.3
1,000	4.63	34.577	71	2.99	35.2
1,150	4.02	34.589	75		35.3
1,900	2.39	34.650	112	2.85	36.5
2,200	2.11	34.656	122	2.77	36.5
2,300	2.12	34.655	115	2.78	35.3
2,400	2.07	34.655	120	2.77	35.3
2,460	2.07	34.669	117	2.78	36.4
Subarctic Pacific					
30	8.24	32.499	305	1.10	29.8
100	4.86	32.893	275	1.54	29.5
200	3.93	33.758	130	1.99	34.4
400	3.79	34.009	60	2.74	33.3
500	3.68	34.126	47	2.58	34.3
600	3.48	34.190	36	2.58	32.8
800	3.13	34.284	29	2.88	34.2
1,000	2.79	34.347	23	2.98	33.8
1,250	2.47	34.419	26	2.93	34.7
1,500	2.21	34.462	36	2.64	34.7
2,000	1.84	34.526	60	2.77	34.2

Samples from MANOP, Site S (11°N, 140°W) were collected in May 1979. Galapagos samples (0°44' N, 86°10' W) were collected in March 1979, and those from the subarctic Pacific (50° N, 150° W) in June 1981.

material is also an important carrier of particulate vanadium. The compositions of fine suspended particulate matter⁴ and material from sediment traps in the deep Atlantic⁵ show excellent correlations between vanadium and aluminium at crustal ratios. Although there was significantly more biogenic and terrigenous particulate matter in the water column during March 1979, the concentration of particulate vanadium was higher during July 1976. The V/Al ratio in the surface sample from the 1976 Galapagos profile was 2.4×10^{-2} , which is more than one order of magnitude higher than the crustal ratio (8.5×10^{-4}). The ratio in the deeper samples dropped rapidly towards the crustal value as the biogenic material was remineralized. The same ratio in the March 1979 samples was near to the crustal value throughout the water column. The V/Al ratios in particles collected from sediment traps in the eastern tropical Pacific and from the underlying sediments (J. Dymond and R. C. unpublished data) are also near the crustal values. The flux to these traps ($\sim 0.7\ \text{nmol V cm}^{-2}\ \text{yr}^{-1}$) also matches the accumulation rate of vanadium in the sediments. This emphasizes the refractory

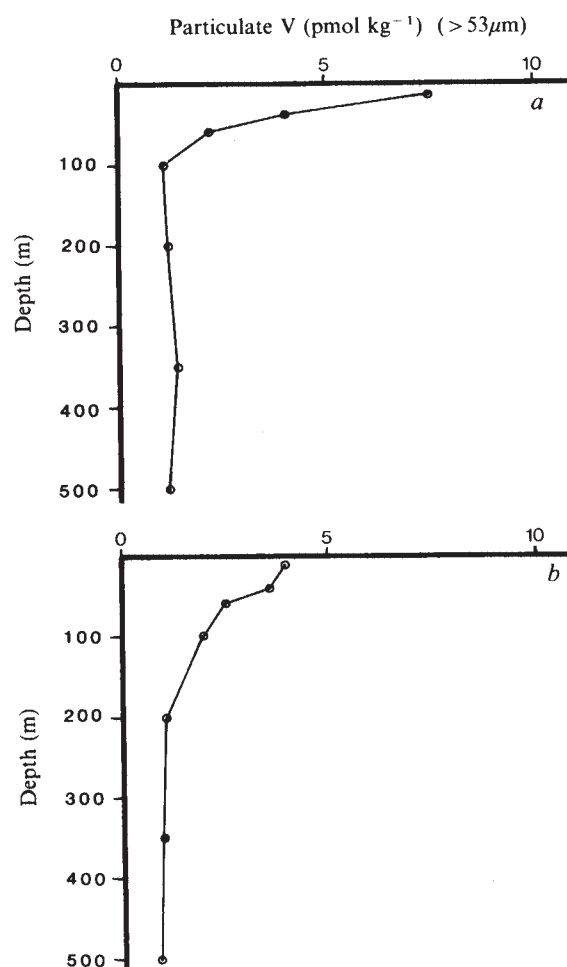


Fig. 1 Concentration of vanadium (pmol V per kg seawater) in particles larger than $53\ \mu\text{m}$. The samples were collected *in situ* by a filtration technique² that separates three particle size fractions (<1 , 1 – 53 , and $>53\ \mu\text{m}$) from large volumes of water (2 – $20\ \text{m}^3$). Thus, the sampling method permits the collection of larger sized particles which, although rare, settle rapidly enough to dominate the vertical mass flux in most environments^{1–3,25}. The size-frequency distribution of the particulate matter on the filters is determined microscopically and, when combined with estimates of settling rates for each particle class, yields an estimate of the total vertical mass flux. This is combined with the bulk chemical composition of the particulate material to estimate the flux of each chemical component. Particulate vanadium was determined by instrumental neutron activation analysis²⁶ of the $>53\ \mu\text{m}$ size fraction. Data shown are samples from the Galapagos site collected in: a, July 1976; b, March 1979.

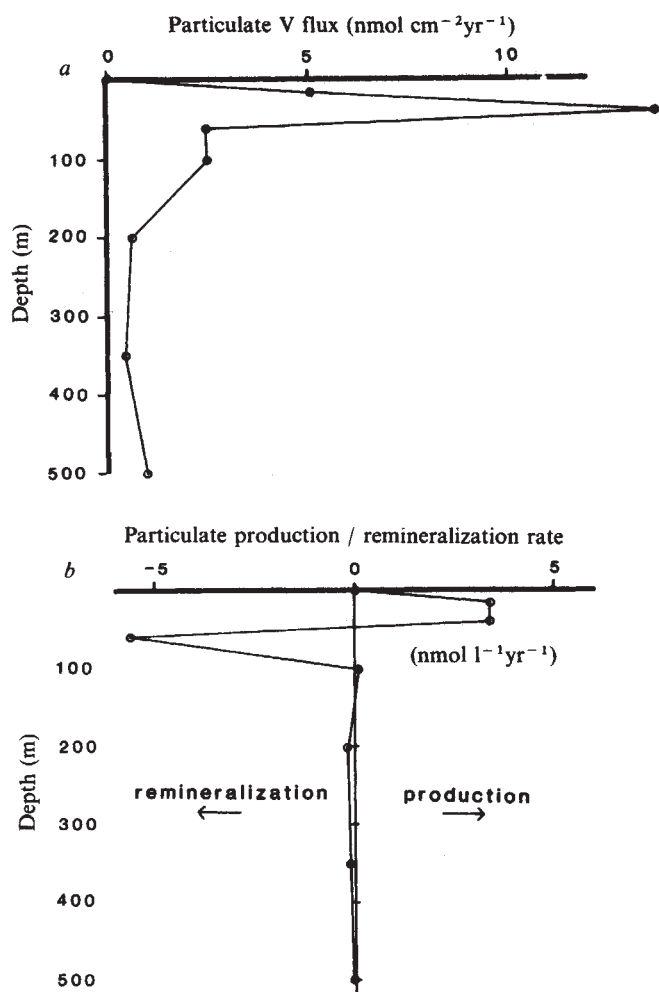


Fig. 2 *a*, Calculated vertical flux of particulate vanadium; *b*, resulting production or remineralization of vanadium due to changes in the particle flux.

nature of the vanadium in these deep samples. Clearly, the 'biogenic' vanadium seen near the surface in the 1976 samples is very labile and does not contribute significantly to the deep flux.

This region of the oceans is subject to large seasonal as well as inter-annual variations in productivity associated with the complex equatorial current systems⁶. The 1976 data were collected during a low productivity event caused by El Niño conditions³ which may have resulted in the observed differences between the two particulate vanadium profiles. The nature of the vanadium association with organic particulate matter is unknown, as is the case with most other trace metals⁷. Although there is a vanadium(III) oxygen carrying protein in some ascidians⁸, its significance to pelagic vanadium cycling is doubtful. There may, however, be labile vanadium enrichments in related organisms such as salps. Other organovanadium complexes (such as tetrapyrroles) found in marine sediments⁹ are stable diagenetic products of porphyrins.

Vertical flux estimates for vanadium from the 1976 profile are shown in Fig. 2*a*. These fluxes are based on the concentration data (Fig. 1*a*) and estimates of the bulk mass flux³. The flux reaches more than $10 \text{ nmol V cm}^{-2} \text{ yr}^{-1}$ at 60 m but drops off by an order of magnitude below 200 m depth. Based on the observed particle compositions³, vanadium behaviour in the upper water column parallels the formation and decomposition of particulate phosphorus at a constant V/P ratio of 4×10^{-3} . Using this ratio with an estimate of the global flux of particulate phosphorus¹⁰ ($0.87 \mu\text{mol P cm}^{-2} \text{ yr}^{-1}$) yields an estimate of the flux of vanadium out of the surface ocean of $3.5 \text{ nmol V cm}^{-2} \text{ yr}^{-1}$.

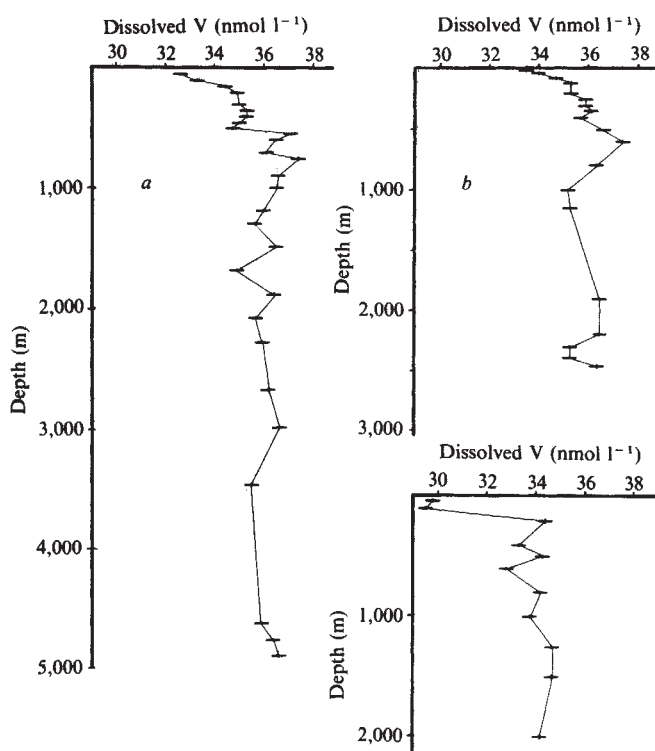


Fig. 3 Dissolved distributions of vanadium showing surface depletions: *a*, MANOP site S; *b*, Galapagos site; *c*, subarctic Pacific. The analysis for dissolved vanadium was made by atomic absorption spectrometry using a graphite furnace atomizer. For pre-concentration of the vanadium, the acidified samples were buffered to pH 4 and the trace elements were co-precipitated with cobalt-APDC¹⁶. The method recovers both V(IV) and V(V) oxy-anions from seawater with an efficiency of 80%. The limit of detection using 35 ml of seawater is 1 nmol V per kg seawater; the precision is 0.4 nM (1σ) at 35 nM vanadium.

Assuming that the vertical profile is in steady state, a change in the flux as a function of depth requires *in situ* production or remineralization of particulate vanadium (Fig. 2*b*). If this change occurs solely through exchange with dissolved vanadium, the estimated fluxes also represent the dissolved depletion or regeneration rate. This rate can be compared with other fluxes in the marine vanadium cycle by using a simple vertical two-box model^{7,11}. Depletion of vanadium from surface waters must be balanced by primary inputs, including riverine and atmospheric inputs as well as physical exchange with deeper water. The riverine¹² and atmospheric^{6,13} inputs probably supply $\leq 0.4 \text{ nmol V cm}^{-2} \text{ yr}^{-1}$ such that at least 85% of the particulate flux estimated from the LVFS data must be supplied by exchange of depleted surface water with the deep ocean. At an average volume exchange rate of 3.5 m yr^{-1} distributed over the ocean surface, the surface water reservoir should be depleted in vanadium by about 8 nmol l^{-1} relative to the deep reservoir.

When these flux estimates were completed¹⁴, very little data existed for the concentration of vanadium in seawater¹⁵, and none had been generated using currently-accepted sampling methods. Especially lacking were any high-quality profiles from the Pacific. Therefore, vanadium was analysed in samples collected from the Pacific specifically for trace metal analysis. The hydrographic data and concentrations of vanadium are given in Table 1 and the distributions are presented in Fig. 3. A surface depletion of V is clearly demonstrated, although the magnitude is about one-half that predicted by the particulate data. The average 'specific' vanadium (normalized to a salinity of 35.0) was 32.7 nM in these Pacific surface waters and 36.4 nM in the deep Pacific. Based on the pelagic data, the residence time of dissolved vanadium in the ocean with respect to its river input¹² is $\sim 5 \times 10^4 \text{ yr}$.

Significant processes affecting the concentration of trace metals at the ocean margins (such as increased scavenging, diffusion from sediments, or inputs from rivers) have been identified through the examination of surface water samples collected on transects moving away from coastal regions¹⁶⁻¹⁸. The specific vanadium concentrations in surface samples taken along a transect off California (at 39° 38' N) had an average value of 32.4 nM (similar to the pelagic values) and showed no systematic change near the coast.

The marine geochemical cycles of metals are often affected by their involvement in redox cycles^{19,20}. Major concentration changes result from direct changes in solubility as a function of oxidation state and scavenging by fresh hydrous metal oxides produced near oxygen interfaces. The specific vanadium concentration within the anoxic zone of Saanich Inlet, British Columbia was ~26 nM which is somewhat lower than the pelagic value. However, there were no systematic changes in the concentration of vanadium with depth that related to other redox parameters.

These pelagic distributions are consistent with other recently

reported determinations of dissolved vanadium²¹⁻²⁴, although a slight surface enrichment has been reported in the western North Atlantic²³. In view of the variability of particulate vanadium seen at the Galapagos site (Fig. 1a, b), it is not surprising that some variations in its dissolved surface depletion exist. The simple box model applied to estimate the surface depletion is, at best, a crude approximation of the oceans and the 1976 data set used to derive the flux (Fig. 2) may not be representative of the mean biogenic flux of vanadium. Therefore, the factor of 2 agreement between the predicted and measured distributions is encouraging. There is certainly much more to learn about the detailed chemistry of vanadium and other metals in the oceans. This investigation demonstrates how both particulate matter and dissolved distributions can be coupled to study these biogeochemical cycles.

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1. Wong, C. S., Boyle, E., Bruland, K. W., Burton, J. D. & Goldberg, E. D. (eds) *Trace Metals in Seawater*, Session VI, 367-536 (Plenum, New York, 1983).
2. Bishop, J. K. B. & Edmond, J. M. *J. mar. Res.* **34**, 181-198 (1976).
3. Bishop, J. K. B., Collier, R. W., Ketten, D. R. & Edmond, J. M. *Deep-Sea Res.* **27**, 615-640 (1980).
4. Buat-Menard, P. & Chesselet, R. *Earth planet. Sci. Lett.* **42**, 399-411 (1979).
5. Brewer, P. G., Nozaki, Y., Spencer, D. W. & Fleer, A. P. *J. mar. Res.* **38**, 703-728 (1980).
6. Wyrki, K. *Int. J. Oceanol. Limnol.* **1**, 117-147 (1967).
7. Collier, R. W. & Edmond, J. M. *Prog. Oceanogr.* (in the press).
8. Carlisle, D. B. *Proc. R. Soc. B171*, 31 (1968).
9. Lewan, M. D. & Maynard, J. B. *Geochim. cosmochim. Acta* **46**, 2547-2560 (1982).
10. Froelich, P. N. thesis, Univ. Rhode Island (1979).
11. Broecker, W. S. *Quat. Res.* **1**, 188-207 (1971).
12. Martin, J. M. & Meybeck, M. *Mar. Chem.* **7**, 173-206 (1979).

13. Duce, R. A., Umami, C. K., Ray, B. J. & Atimoto, *Trans. Am. geophys. Un. Abstr.* **63**, 987 (1982).
14. Collier, R. W. & Edmond, J. M. *Trans. Am. geophys. Un. Abstr.* **61**, 1007 (1980).
15. Moris, A. W. *Deep-Sea Res.* **22**, 49-54 (1975).
16. Boyle, E. A., Huested, S. S. & Jones, S. P. *J. geophys. Res.* **86**, 8048-8066 (1981).
17. Nozaki, Y., Thomson, J. & Turekian, K. K. *Earth planet. Sci. Lett.* **32**, 304-312 (1976).
18. Bruland, K. W. & Franks, R. P. in *Trace Metals in Sea Water* (eds Wong, C. S., Boyle, E., Bruland, K. W., Burton, J. D. & Goldberg, E. D.) 395-414 (Plenum, New York, 1983).
19. Spencer, D. W. & Brewer, P. G. *J. geophys. Res.* **76**, 5877-5892 (1972).
20. Emerson, S., Cranston, R. E. & Liss, P. S. *Deep-Sea Res.* **26**, 859-878 (1979).
21. Collier, R. W. *Trans. Am. geophys. Un. Abstr.* **63**, 989 (1982).
22. Zhou, J.-Y., McDuff, R. & Murray, J. W. *Trans. Am. geophys. Un. Abstr.* **63**, 989 (1982).
23. Huizenga, D. L. & Kester, D. R. *Trans. Am. geophys. Un. Abstr.* **63**, 990 (1982).
24. Martin, J. H. & Knauer, G. A. *Trans. Am. geophys. Un. Abstr.* **63**, 960 (1982).
25. McCave, I. N. *Deep-Sea Res.* **22**, 491-502 (1975).
26. DeSoete, D., Gijbels, R. & Hoste, J. *Neutron Activation Analysis, chem. analyt. Ser.* **34**, (1972).

Radium fluxes from a salt marsh

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Dissolved ²²⁶Ra (half life 1,600 yr), ²²⁸Ra (half life 5.7 yr) and ²²⁴Ra (half life 3.64 days) activities in estuarine and coastal waters higher than values reported for open ocean or river waters have been explained by desorption and diffusion of radium from coastal and estuarine sediments¹⁻⁴. Fluxes of radium isotopes from sediments in Long Island Sound, the Hudson River estuary, Chesapeake Bay and Winyah Bay, South Carolina, have been estimated from mass balances, diffusive bottom fluxes and desorption from suspended sediments⁴⁻⁷. These estimates may not be correct if salt marshes which are intimately associated with estuaries and the coastal ocean are an additional source or sink of radium⁴. In this study Bly Creek, a small marsh-tidal creek system near Georgetown, South Carolina, was chosen to determine the flux of ²²⁸Ra and its great granddaughter, ²²⁴Ra, from the sediments to the tidal creek water. We find that the highest activities of ²²⁴Ra and ²²⁸Ra in the tidal creek waters occur during low tide, but ²²⁶Ra activity remains relatively constant throughout the tidal cycle. Fluxes of ²²⁴Ra and ²²⁸Ra calculated from mass balances required to support the observed activities in the creek waters and measured directly using a flux chamber are consistent. These measurements reveal a large input of radium from the marsh sediments to the overlying waters. To produce the necessary fluxes, irrigation and bioturbation must replace interstitial water in the top 5 cm of the marsh sediments every 2-3 days.

Surface water samples collected at approximately 40-min intervals over two tidal cycles were processed for radium isotope determinations⁸. Water velocity and depth were measured simultaneously. The activity ratios ²²⁸Ra/²²⁶Ra and ²²⁴Ra/²²⁶Ra were determined by γ -ray spectrometry and absolute ²²⁶Ra activities

were determined by radon emanation^{3,4}. Absolute ²²⁴Ra and ²²⁸Ra activities were calculated from the activity ratios and the absolute ²²⁶Ra activities. ²²⁶Ra activities remained constant and net water flux was essentially zero throughout the study.

The ²²⁴Ra and ²²⁸Ra data describe two straight lines when plotted against the height of water at the sampling site in Bly Creek (Figs 1, 2). The highest activities of radium occur during the lowest tide heights. We conclude that this linear relationship between water depth and radium activity must represent dilution of high-activity marsh waters with low-activity ocean water. Thus, radium must be supplied to the creek waters from the marsh sediments to maintain the high activities observed at low tide. The only other potential source, groundwater, can be excluded because salinities did not vary significantly in Bly Creek during the study.

If Ra in the marsh system is in steady state, that is, if the Ra exported during ebb tide is balanced by production and release of Ra from marsh sediments, we can use mass balances to determine Ra fluxes from the sediments.

$$F_{\text{Ra}} = \frac{V_{\text{out}}\text{Ra}_{\text{out}} - V_{\text{in}}\text{Ra}_{\text{in}}}{A\Delta t} \quad (1)$$

where F_{Ra} is the flux of Ra from the sediment; V_{out} is equal to V_{in} , the volume of water exchanged during one tidal cycle; Ra_{out} is the Ra exported from the marsh (d.p.m. l⁻¹); Ra_{in} is the Ra imported from the coastal ocean (d.p.m. l⁻¹); A is the area of marsh flooded at high tide; and Δt is the time in one tidal cycle (12 h).

Measurements of Ra isotopes in nearby coastal waters reveal that activities of ²²⁴Ra and ²²⁸Ra are quite low (of the order of 0.2-0.3 d.p.m. l⁻¹)^{8,9} and for a first approximation can be neglected when compared with the activities observed in the marsh waters (1-3 d.p.m. l⁻¹). Thus, equation (1) simplifies to

$$F_{\text{Ra}} = \frac{V_{\text{out}}\text{Ra}_{\text{out}}}{A\Delta t}$$

Topographic data are used to estimate the volume of water

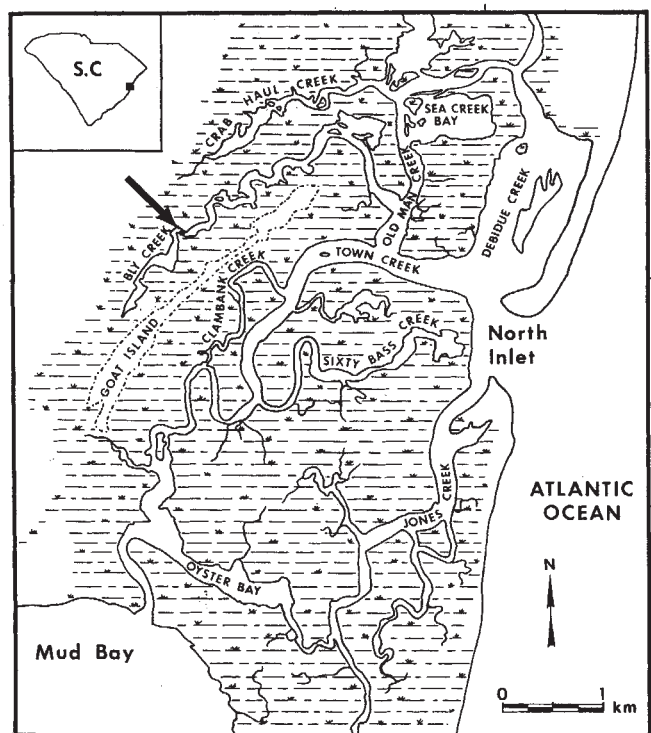


Fig. 1 Map of marsh surrounding North Inlet, South Carolina. Location of primary sampling site on Bly Creek (33°20' N 79°12' E) is indicated by the arrow. Bly Creek drains the sandy sediments between ancient beach ridges, one of which is labelled Goat Island.

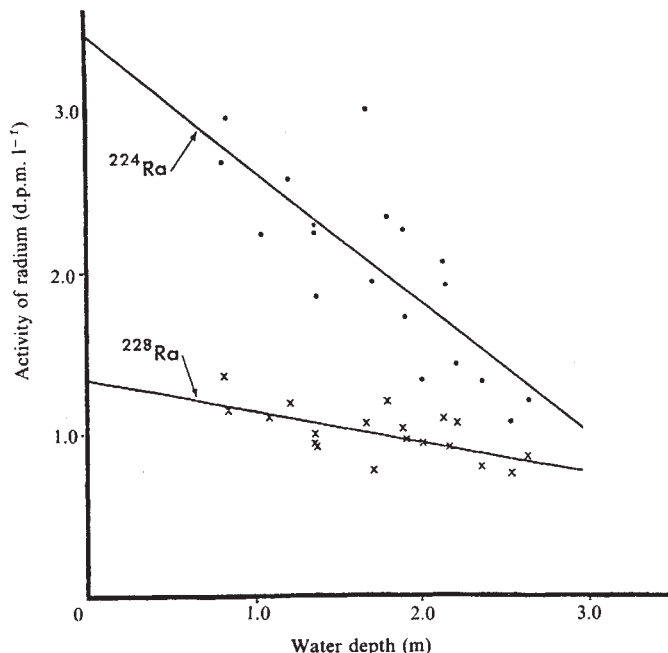


Fig. 2 The activity of radium versus the water depth in Bly Creek on 15 and 16 October 1982. Correlation coefficients for ^{224}Ra and ^{228}Ra are -0.795 and -0.667 , respectively.

leaving the marsh on the ebb tide, V_{out} . The average activity of radium over one tidal cycle, R_{out} , is $2.1 \text{ d.p.m. l}^{-1}$ for ^{224}Ra and $1.0 \text{ d.p.m. l}^{-1}$ for ^{228}Ra (Fig. 2). Thus, the fluxes of ^{224}Ra and ^{228}Ra are $2 \times 10^{-7} \text{ d.p.m. cm}^{-2} \text{ s}^{-1}$ and $1 \times 10^{-7} \text{ d.p.m. cm}^{-2} \text{ s}^{-1}$, respectively.

To determine whether these fluxes are realistic, we devised a flux chamber consisting of a 25-cm diameter, 1.4-m long polyvinylchloride pipe closed at one end except for an air vent. The open end of the chamber is inserted about 15 cm into the sediment. An opening just above the sediment is connected by flexible hose to a conveniently located column which is secured near the sediment surface and filled with acrylic fibre impregnated with manganese dioxide (Mn-fibre)¹⁰. The apparatus is installed at low tide in an area free of surface water; as the tide rises, water passes through the Mn-fibre column to fill the chamber. The Mn-fibre strips all radium isotopes from the incoming water. At high slack tide the Mn-fibre column is replaced with a fresh Mn-fibre column. As the tide falls, any radium which may have moved from the sediment into the chamber is collected by the Mn-fibre column as water drains from the chamber. Fluxes of ^{224}Ra and ^{228}Ra obtained by this method are $6 \times 10^{-7} \text{ d.p.m. cm}^{-2} \text{ s}^{-1}$ and $2 \times 10^{-7} \text{ d.p.m. cm}^{-2} \text{ s}^{-1}$, respectively.

The fluxes calculated from the tidal cycle data agree with the fluxes measured by the chambers to within a factor of 3. Considering the assumptions used in the tidal cycle calculation and possible variation in fluxes throughout the marsh, this agreement is very good.

Decay of ^{228}Th (half life 1.9 yr) produces ^{224}Ra in the marsh sediments. Using $0.5 \text{ d.p.m. } ^{228}\text{Th cm}^{-3}$ of sediment (based on initial analyses) gives a production term of $5.9 \times 10^{-6} \text{ d.p.m. } ^{224}\text{Ra cm}^{-2} \text{ s}^{-1}$ over a depth of 5 cm. We chose 5 cm as the depth from which ^{224}Ra can be derived because: (1) this is approximately the top of the dense root zone of the *Spartina*, and (2) preliminary measurements show ^{224}Ra in interstitial waters to be depleted in the top 5 cm but constant below 5 cm. Comparison of this potential source with the flux necessary to support the ^{224}Ra in the water column (from the more conservative tidal

cycle data) reveals that only about one-third of the ^{224}Ra produced in the top 5 cm of sediment need be mobilized to yield the required fluxes. A similar calculation for ^{228}Ra reveals that to support the observed flux, ^{228}Ra must be removed from at least 25 cm of sediment if this is a steady state flux. However, the ^{228}Ra flux may be periodic with seasonal gains and losses of the longer-lived Ra isotopes in the sediments.

Three mechanisms may be driving this exchange process: (1) molecular diffusion, (2) advection of interstitial water from the sediments due to turbulence in the overlying water and tidal pumping, and (3)urbation and irrigation by organisms. To estimate the scale length (L) over which molecular diffusion is effective we use the relationship $L = \sqrt{D/\lambda}$ where D is the effective diffusion coefficient of Ra, $3.4 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ (assuming a temperature of 18 °C, a tortuosity of 2.0, and an adsorption coefficient of 100)¹¹⁻¹³, and λ is the decay constant of ^{224}Ra , $2.2 \times 10^{-6} \text{ s}^{-1}$. Thus, the path length is 0.12 cm. Our preliminary interstitial measurements indicate that ^{224}Ra is removed from at least 40 times this depth; therefore, other factors must be increasing the exchange. Because the flux chambers show Ra fluxes almost as high as the tidal cycle data require and as the closed chambers inhibit physical turbulence, mixing due to turbulence of interstitial water is probably not the major transport mechanism of radium from the marsh sediments. In addition, a preliminary experiment indicates that a significant amount of water is not entering the chamber due to tidal pumping.

However, bioturbation and irrigation may indeed increase the flux of radium from the sediments by increasing the effective surface area of the marsh and by physically moving water of higher radium activity to the overlying water. Fiddler crab burrows dot the sediment surface from the creek banks to the low *Spartina alterniflora*, *Salicornia* areas of the marsh and effectively increase the sediment surface area. Worms, fiddler crabs and other residents of the marsh sediments also rework the sediments, physically moving water containing high radium activity towards the surface of the marsh.

The activities of ^{224}Ra in the water at low tide ($C_L = 3 \text{ d.p.m. l}^{-1}$) and high tide ($C_H = 1 \text{ d.p.m. l}^{-1}$) and in the pore water at 5 cm depth ($C_P = 12 \text{ d.p.m. l}^{-1}$) as well as the volume of water which flushes the marsh during one tidal cycle

$V_{in} = V_{out}$) constrain the volume of water which is mixed through the upper 5 cm of the sediment (V_{mix}) as follows:

$$V_{out} C_L = V_{in} C_H + V_{mix} C_P - V_{mix} C_H$$

Using the observed data $V_{mix} = 9 \times 10^9 \text{ cm}^3$. Thus, the residence time of water in the upper 5 cm of sediment can be determined from the following equation

$$\tau = \frac{\text{porosity} \times \text{depth} \times \text{area}}{V_{mix}/12 \text{ h}}$$

and is of the order of 2–3 days.

Our study indicates that salt marshes are important sources of radium isotopes to coastal waters. The short residence time of water in the top centimetres of the salt marsh sediments and the high flux of radium out of the sediments demonstrate that marshes are extremely active chemical systems able to respond to changing conditions on a time scale of days. These observations have important implications for our understanding of the cycling of nutrients, metals, carbon and pollutants in coastal systems.

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1. Moore, W. S. *Earth planet. Sci. Lett.* **2**, 232–234 (1969).
2. Blanchard, R. L. & Oakes, D. J. *geophys. Res.* **70**, 2911–2921 (1966).
3. Elsing, R. J. & Moore, W. S. *Earth planet. Sci. Lett.* **48**, 239–249 (1980).
4. Elsing, R. J. & Moore, W. S. *Earth planet. Sci. Lett.* **64**, 430–436 (1983).
5. Thomson, J., Turekian, K. K. & McCaffrey, R. J. in *Estuarine Research Vol. 1* (ed. Cronin, L. E.) 28–44 (Academic, New York, 1975).
6. Cochran, J. K. thesis Yale Univ. (1979).
7. Li, Y.-H. & Chan, L. H. *Earth planet. Sci. Lett.* **43**, 343–350 (1978).
8. Elsing, R. J., King, P. T. & Moore, W. S. *Analyt. chim. Acta* **114**, 277–281 (1982).
9. Levy, D. M. thesis Univ. South Carolina (1983).
10. Moore, W. S. *Deep Sea Res.* **23**, 647–651 (1976).
11. Li, Y.-H. & Gregory, S. *Geochim. cosmochim. Acta* **38**, 703–714 (1974).
12. Berner, R. A. *Early Diagenesis—A Theoretical Approach*, 241 (Princeton University Press, 1980).
13. Cochran, J. K. & Krishnaswami, S. *Am. J. Sci.* **280**, 849–889 (1980).

Ultraviolet sensitivity in hyperpolarizing photoreceptors of the giant clam *Tridacna*

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The giant clam *Tridacna* is a common shallow-water inhabitant of Indo-Pacific coral reefs¹. These animals contain large populations of photosynthetic zooxanthellae in hypertrophied siphonal lobes which extend well beyond the edge of the shell for maximum exposure to light^{2–4} (Fig. 1). Protection for these large fleshy lobes is visually mediated and consists of a partial siphon retraction and valve adduction, a behaviour which gives rise to a forceful, startling jet of seawater⁵. Numerous eyes (up to several thousand in large animals⁶) located along the margin of the siphon comprise a visual system which presumably mediates the defensive withdrawal reflex. Here we present the first report on the physiological properties of the visual system in *Tridacna*. Intracellular electrophysiological recordings have revealed two distinct types of retinal cells: both are hyperpolarized by light but only one generates axonal spikes. We also describe cells with differential colour sensitivity, including receptors that are sensitive to ultraviolet (UV) light. Ultraviolet sensitivity, although not previously reported in molluscs⁷, may be an adaptation in *Tridacna* to the environmental requirements of a host for algal symbionts.

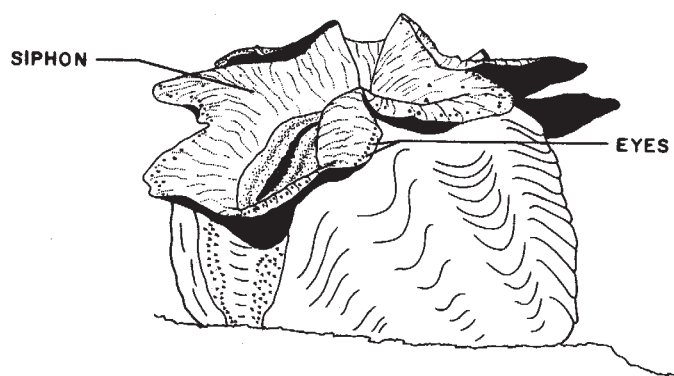


Fig. 1 *Tridacna maxima* L., drawn from a 35 mm colour slide projection (see cover) showing the posterior lobes of the siphon, the slit-like inhalant aperture and the right valve. The siphon lobes are fully extended, as they are in bright sunlight, with the eyes (bold dots) forming a line around the lateral margins. Shell length, 16.5 cm.

Tridacna were collected at Davies Reef in northern Queensland. The eyes are visible at the mantle surface as clear lens-like structures surrounded by a dense accumulation of pigmented zooxanthellae. Retinal cells line the back of the eye capsule and an optic nerve extends into the adjacent tissues^{3,8}. In these experiments the epithelium was removed and the lens gently expressed to expose the retina. Glass micropipettes containing 3 M KCl were then lowered directly onto the retina for intracellular recording. Light from a xenon arc source was shone on the eye using a fibre optic light guide and was controlled by an electronic shutter in combination with neutral density and interference filters.

In recordings from over 100 light-sensitive cells, the receptor potentials were exclusively hyperpolarizing (Fig. 2). However, two distinct classes of cells exist: spiking (S) and non-spiking (NS). Of the cells encountered in these experiments, a slight majority were NS (52%), although both types were observed in most eyes. That the absence of spikes in NS cells is a physiological characteristic of these receptors, and not an artefact due to injury from electrode penetration, was established by the following observations: (1) injury discharges, which were occasionally observed in S cells, were never seen in NS cells; (2) S-cell spikes remain for the duration of stable penetrations in most instances, occasionally lasting for 30–60 min; and (3) additional physiological characteristics invariably correspond with the presence or absence of spikes. For example, NS cells have low resting membrane potentials in the dark ($-14.4 \pm 6.7 \text{ mV}$, $\pm \text{s.d.}$) and large receptor potential amplitudes (Fig. 2a); responses greater than 100 mV have been observed in fully dark-adapted preparations, but these potentials are readily attenuated by successive light stimuli and adapt to an intermediate level of polarization ($-37.8 \pm 12.7 \text{ mV}$) under steady illumination at high intensities (Fig. 2b). In contrast, S cells have a larger resting membrane potential ($-43.8 \pm 6.6 \text{ mV}$) than NS cells, but the receptor potential amplitude is smaller ($14.0 \pm 5.2 \text{ mV}$) and not affected by light or dark adaptation. The difference in adaptation sensitivity is most striking in response to light flashes of long duration where, by comparison with NS cells, the S cell maintains a steady level of membrane hyperpolarization; in some instances there is a subsequent gradual depolarization by a few millivolts (for example, 3–4 mV in Fig. 2c). However, there was very little difference in response latency between S and NS cells.

As with the cell represented in Fig. 2c, many S cells are spontaneously active in the dark and the spikes, 3–8 mV in amplitude, are inhibited by light. In response to dimming, bursts of spikes (60–80 Hz; 0.5–2.0 s) are generated on the depolarizing phase of the receptor potential, with the frequency and duration of the burst being increased by longer periods of light exposure. Thus, S cells are sensitive to light adaptation, although not directly in terms of the receptor potential waveform.

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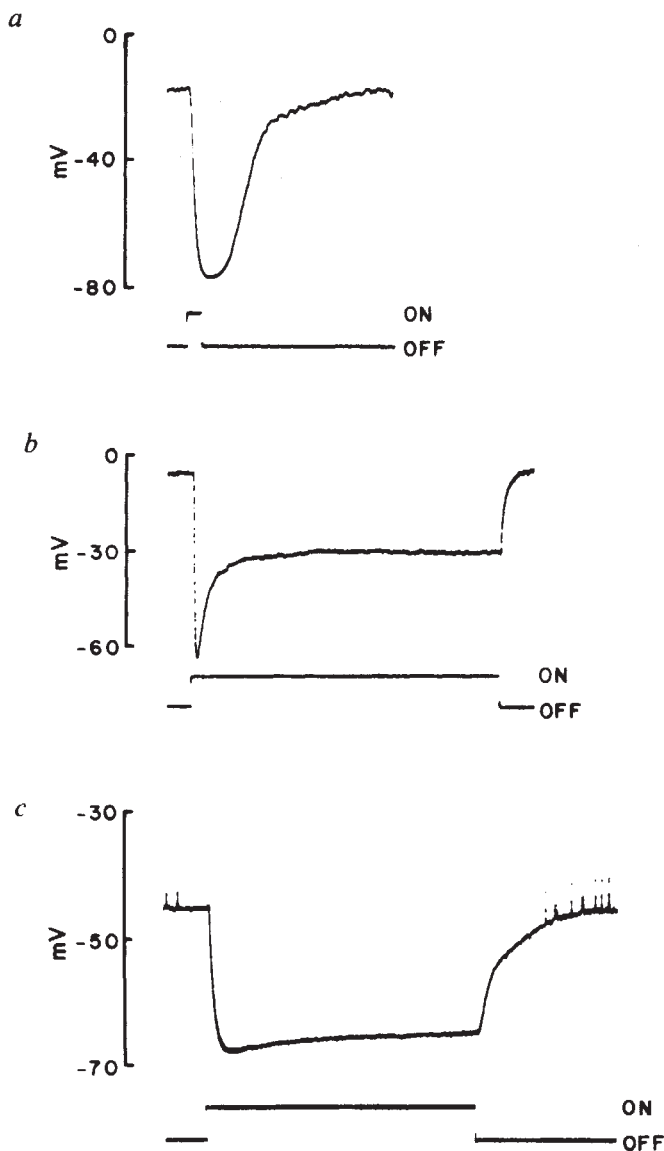


Fig. 2 Hyperpolarizing receptor potentials recorded intracellularly from *Tridacna* retinal cells. Receptor potentials from non-spiking (NS) photoreceptors in response to *a*, a brief light flash (250 ms); *b*, a light-adapting stimulus (2 min). NS receptors have a low resting potential in the dark and adapt rapidly to light. *c*, Receptor potential from a spiking (S) photoreceptor in response to an extended period of light stimulation (1.5 min). S receptors have higher resting potentials than NS cells but the light-mediated hyperpolarization does not adapt to light. Spikes occur spontaneously and in bursts on the depolarizing phase at light-off.

To test the colour sensitivity of *Tridacna* photoreceptors, receptor potential amplitudes were measured in response to monochromatic light flashes from 350 to 610 nm. Data were used only from experiments in which stable responses were obtained for V -log I test sequences preceding and following the spectral test series. Surprisingly, we found individual receptors with sensitivity peaks at three specific wavelengths (Fig. 3): of 13 cells meeting the criteria, seven were green-sensitive ($\lambda_{\max} = 490$ nm), two were blue-sensitive ($\lambda_{\max} = 450$ nm), and four were sensitive to UV light ($\lambda_{\max} = 360$ nm). All of the NS cells in this group were sensitive to green light, whereas S cells were represented in each category including one green- and all of the blue- and UV-sensitive photoreceptors. Behavioural sensitivity to various colours of light was also tested. Under a low level of white background illumination, siphon retractions were

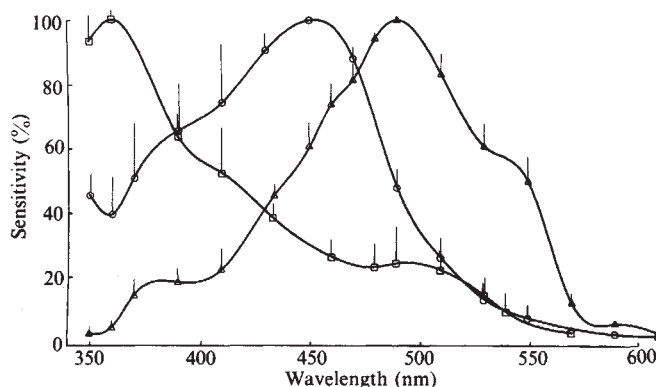


Fig. 3 Spectral sensitivity of *Tridacna* photoreceptors. Relative sensitivity, as measured by the amplitude of the hyperpolarizing receptor potential, plotted against wavelength. Individual photoreceptor sensitivity curves fall into three groups and the results shown are the means (± 1 s.e.) for cells in each category with curve-smoothing based on a spline function: $\lambda_{\max} = 490$ nm, $n = 7$ (Δ); $\lambda_{\max} = 450$ nm, $n = 2$ ($\circ$); $\lambda_{\max} = 360$ nm, $n = 4$ ($\square$). Constant intensity light flashes, of 100–200 ms duration in different experiments, were given at 15-s intervals—stimulus parameters determined not to be light adapting.

recorded in response to 3-s interruptions of adapting light at selected wavelengths. The siphon responded to conditioning light over the range of colours used in the physiological studies, but the threshold intensity for UV light (350 nm) was approximately four times greater than that for green light (490 nm).

Tridacna photoreceptors exhibit several interesting physiological features. In contrast to the scallop whose eyes contain both depolarizing (proximal) and hyperpolarizing (distal) photoreceptors⁹, each directly initiating axon spikes, *Tridacna* has two types of hyperpolarizing receptor but only one has the ability to generate spikes. It is possible that *Tridacna* NS cells may not be photoreceptors, but instead are glial cells passively responding to adjacent receptors. This explanation is unlikely as (1) other cells (glial candidates) with no response of any kind are frequently penetrated, (2) response latencies are equivalent for both receptor cells, and (3) Lucifer yellow injections of both S and NS cells show axons projecting from the cell soma (unpublished results). Non-spiking photoreceptors, in addition to the vertebrate rods and cones, are now well known in arthropods (for example, barnacles¹⁰) but they have not previously been reported in molluscs and their physiological role is unknown. The S cell, on the other hand, is the physiological equivalent of the scallop distal photoreceptor: common features include a hyperpolarizing receptor potential which does not adapt to light⁹, and an 'off-response' burst of spikes, the intensity of which is directly related to preceding light conditions¹¹. Spike output therefore appears to correspond to an accumulating excitatory component of the light response which is masked by a separate 'primary' light-inhibiting effect expressed by the hyperpolarizing receptor potential. It has been suggested¹² that primary light inhibition may be characteristic of bivalve visual systems where the predominant light response in a variety of pallial photoreceptor systems is to dimming, that is, to a shadow^{12–14}, and where behaviourally relevant shadow reflexes are the norm^{12,15}.

Of even greater interest is the existence of receptors sensitive to short wavelengths of light, specifically to UV. In previous studies encompassing each of the three major molluscan classes^{7,12,13}, the maximum sensitivity for rhodopsin-like photopigments was typically around 490 nm, a value corresponding with that of the NS cells in *Tridacna*. UV light receptors represent a significant departure from this pattern, and it is doubtful that the electroretinograph or compound nerve-recording techniques used in several of the previous studies would have obscured the presence of UV receptors. *Tridacna* is a unique mollusc in several

respects, and recognition of these features can assist in the formulation of hypotheses to account for UV sensitivity in this genus.

Of the previous molluscs tested, including several intertidal bivalves, all are able to limit their exposure to UV light by means of protective shells or cryptic locomotor behaviour, and/or they occupy temperate neritic waters where this spectral component is rapidly absorbed at the surface. In contrast, *Tridacna* occurs in a shallow-water tropical habitat, including reef flats that are partially exposed at low tide, where turbidity is low and UV penetration maximal^{16,17}. In these regions UV irradiation is known to be a major limiting factor in the distribution of marine invertebrates¹⁸. Despite this fact, *Tridacna* intentionally exposes its siphonal lobes to intense light and furthermore does not normally withdraw completely into its shell unless provoked repeatedly. UV light receptors may somehow be involved in this positive siphonal phototropism, which in turn may be correlated with the fact that the algal symbionts use a broad spectrum of

light (300–700 nm) for photosynthesis¹⁹. Alternatively, the UV receptors may be functional components of the defensive withdrawal reflex, a behavioural possibility established in the present experiments and further supported by the fact that UV sensitivity is represented in the class of receptors that are physiologically adapted to respond directly to shadows. Whatever the precise role, it seems that *Tridacna* has adapted a range of spectral sensitivities within its population of retinal cells to match the colours present in its environment.

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1. Rosewater, J. *Indo-Pacif. Mollusca* 1, 347–396 (1965).
2. Yonge, C. M. *Scient. Rep. Gi Barrier Reef Exped.* 1, 283–321 (1936).
3. Yonge, C. M. *Rec. aust. Mus.* 33, 735–777 (1980).
4. Trench, R. K., Wetthey, D. S. & Porter, J. W. *Biol. Bull.* 161, 180–198 (1981).
5. Stasek, C. R. *Veliger* 8, 29–35 (1965).
6. Fankboner, P. V. *Veliger* 23, 245–249 (1981).
7. Menzel, R. *Handbook of Sensory Physiology* Vol. VII/6A (ed. Autrum, H.) 503–580 (Springer, Berlin, 1979).
8. Stasek, C. R. *Calif. Acad. Sci. occ. Pap.* 58, 1–9 (1966).

9. McReynolds, J. S. & Gorman, A. L. F. *J. gen. Physiol.* 56, 376–391 (1970).
10. Shaw, S. R. *J. Physiol., Lond.* 220, 145–175 (1972).
11. Hartline, H. K. *J. cell comp. Physiol.* 11, 465–478 (1938).
12. Kennedy, D. *J. gen. Physiol.* 44, 277–299 (1960).
13. Wiederhold, M. L., MacNichol, E. F. Jr & Bell, A. L. *J. gen. Physiol.* 61, 24–55 (1973).
14. Barber, V. C. & Land, M. F. *Experientia* 23, 677–678 (1967).
15. Land, M. F. *Symp. zool. Soc. Lond.* 23, 75–96 (1968).
16. Jerlov, N. G. *Nature* 166, 111–112 (1950).
17. Smith, R. C. & Baker, K. S. *Photochem. Photobiol.* 29, 311–323 (1979).
18. Jokiel, P. L. *Science* 207, 1069–1071 (1980).
19. Halldal, P. *Biol. Bull.* 134, 411–424 (1968).

Threshold channels—a novel type of sodium channel in squid giant axon

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Sodium channels in nerve and muscle cells are functionally similar across wide phylogenetic boundaries¹ and are usually thought to represent a single, homogeneous population that initiates the action potential at threshold and unerringly transmits it along the surface membrane. In marked contrast, many cell types are known to have several distinct potassium permeability systems^{2,3}. Distinguishable populations of Na channels have been reported in a few cell types, however, including denervated skeletal muscle⁴, embryonic cardiac muscle⁵, Purkinje cell somata⁶ and non-myelinated axons at low temperature⁷. We report here that in squid giant axon, in standard experimental conditions, there are two functionally distinct populations of Na channels. The newly discovered population accounts for only a few per cent of the total Na permeability. The channels are selectively activated by small depolarizations and have very slow closing kinetics. Because these channels activate at voltages near the resting potential and tend to stay open for long times, they must dominate behaviour of the axon membrane in the threshold region for action potential initiation.

Experiments on *Loligo pealei* were performed at the Marine Biological Laboratory, Woods Hole, Massachusetts. Cleaned axons were internally perfused and voltage-clamped as described previously^{8,9}. Improvements in the electronic apparatus were important to this study in allowing the resolution of very small ionic currents ($<1 \mu\text{A cm}^{-2}$). For measuring Na current (I_{Na}) plus gating current (I_g), the external solution contained (in mM): 60 (or 120) NaCl, 50 CaCl₂ and 352 (or 452) Tris pH 7.0 (Sigma) (60 or 120 Na; see figure legends). Gating

currents were recorded by replacing all Na with Tris pH 7.0 and adding 200 nM tetrodotoxin (TTX). I_{Na} records were subsequently generated by subtraction. The internal solution was K-free; both tetramethylammonium (TMA) and Cs were used as K substitutes with similar results (150 mM as glutamate salt and 50 mM as fluoride salt). Internal solutions also contained (in mM): 10 Tris pH 7.0 and 560 sucrose. Pronase (Sigma type XIV) was internally applied (0.5 mg ml^{-1}) in some experiments to destroy inactivation as indicated below. Temperature was 8 °C, holding potential -80 mV , and we compensated for $2\text{--}3 \Omega \text{ cm}^2$ of series resistance. Particular care was taken to test for contamination of I_{Na} by end effects; tests included applying TTX to the axon ends in the air gaps, reducing the external Na concentration to as low as 30 mM, and comparing currents measured from different regions of the same axon. The phenomena described below were not affected by these control procedures.

The new channels that are activated in the threshold region were first recognized by their unusually slow closing kinetics. In Fig. 1a, the trace obtained in 120 mM Na shows the activation of $I_{\text{Na}} + I_g$ during a pulse to -50 mV as Na channels open, and the decay of current when the voltage is returned to -80 mV as the channels close. The tail current at -80 mV clearly has a fast and a slow phase of decay. The same procedure in the absence of external Na (0 Na, no TTX) shows an outward I_g transient at the beginning of the pulse which has the same initial amplitude as in 120 mM Na. After the return to -80 mV , I_g is inward and fast. The slow component is not seen in 0 Na and thus can be identified as Na current. Other experiments also showed it to be TTX-sensitive. Subtraction of the traces in Fig. 1a yields I_{Na} (Fig. 1b), and two components of tail current differing in decay rate by roughly an order of magnitude are still evident. Similar results were seen in every axon (total of 17) in which this phenomenon was examined over a 3-yr period.

Fast and slow components of I_{Na} decay accompany a variety of activating pulse amplitudes and durations. Following large pulses, however, the slow component constitutes a tiny fraction of the total current, and perhaps for this reason has been previously overlooked. Figure 2a illustrates the decay of I_{Na} in a Pronase-treated axon after a small pulse (-40 mV , 15 ms) and after a large pulse activating nearly all of the Na conductance ($+40 \text{ mV}$, 3 ms). The two traces have very different initial ampli-

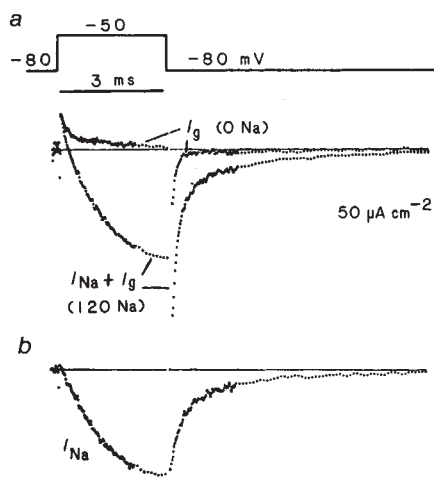


Fig. 1 Time course of Na current (I_{Na}) and gating current (I_g) activation at -50 mV, and deactivation at -80 mV. *a*, $I_{Na} + I_g$ (120 mM external Na) accompanying a 3-ms pulse is compared with I_g alone (0 Na). *b*, I_{Na} generated by subtraction of the traces in *a*. I_{Na} has clear fast and slow components of deactivation. JN243G; 120 mM Na, 200 mM TMA; Pronase-treated.

tudes, but they converge between 1.5 and 2 ms and decay slowly thereafter. Ignoring the small slow component, the $+40$ mV tail can be fitted well by a single exponential with time constant $150 \mu s$. The -40 mV trace can be fitted with the sum of two exponentials having time constants of $180 \mu s$ and 1.9 ms—this disparity in values indicates distinct fast and slow phases of I_{Na} decay.

Figure 2b shows tail currents for a series of activating voltages. Traces for the three largest pulses (-45 , -40 and $+40$ mV) have been truncated to allow the slow tail current to be plotted at high gain. Durations of the pulses ensured that the amplitude of the slow component was maximal in each case. The slow component increases with activating voltage up to ~ -40 mV, where it reaches its maximum size. Its amplitude after the $+40$ mV step is the same. Activation kinetics of the channels underlying the slow tails are voltage-dependent and become faster at more positive voltages. Thus, 3 ms at $+40$ mV is as effective as 10–15 ms at -40 mV.

Each trace in Fig. 2b has both a fast and slow decay phase, but the two components differ greatly in relative magnitude, depending on the activating voltage. For the -55 mV pulse, the fast and slow components are roughly equal in size. Above -40 mV, only the fast component continues to grow, and it is much larger than the slow one after a step to $+40$ mV (Fig. 2a).

These results are very difficult to explain on the basis of a single, homogeneous population of Na channels having multiple conducting states. Instead, they support the existence of a small group of Na channels that are activated over a relatively negative voltage range and have very slow closing kinetics. The fast component of Na tail current reflects activation of a much larger group of 'normal' channels that have fast closing kinetics and activate at more positive voltages. Figure 3 shows the fraction of open channels plotted as a function of membrane potential for both populations. Near the resting potential the percentage of slowly closing channels activated (circles) is far larger than the activated fraction of normal channels. Small depolarizations to the region of action potential threshold (that is, -60 to -55 mV) would selectively activate the new channels. Although the total permeability contributed by this population is small ($\sim 3\%$ of the total in Fig. 3), the channels can carry enough current to depolarize the membrane far beyond threshold for an action potential. Our results suggest that at least half of the channels that are open at threshold (for example, -55 mV in Fig. 2b) are of the new type. Thus, we label them 'threshold' channels, as they must dominate behaviour in the threshold region.

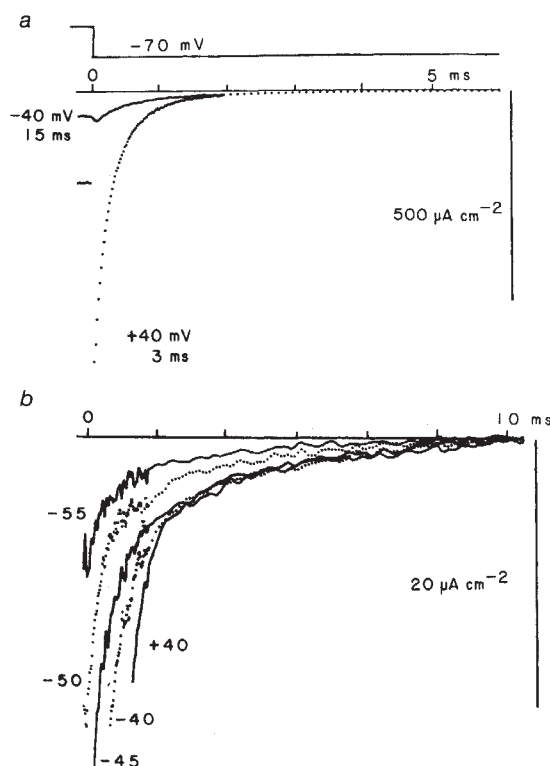


Fig. 2 Behaviour of fast and slow components of Na tail currents as a function of activating voltage. *a*, Currents recorded at -70 mV following a small (to -40 mV, for 15 ms) or large ($+40$ mV, 3 ms) depolarization. The axon was Pronase-treated. The initial amplitude of the tail currents is proportional to the number of channels opened. At -40 mV the number of open channels is $\sim 10\%$ of that at $+40$ mV. Although initial amplitudes are very different, the two traces converge between 1.5 and 2 ms. *b*, Tail currents for a series of activating voltages showing that the amplitude of the slow component saturates at ~ -40 mV. Early portions of the traces for the three largest pulses are truncated; initial amplitudes were (in $\mu A cm^{-2}$) 40.5 (-45 mV), 69 (-40 mV) and 599 ($+40$ mV). Pulse durations were 15 ms, except for 3 ms at $+40$ mV. JN223H; 120 mM Na, 200 mM Cs.

The points for threshold channels in Fig. 3 can be adequately fitted by a two-state model having one open and one closed state. The solid curve assumes that the equilibrium constant for the transition from open to closed varies exponentially with voltage, changing e-fold in 4.5 mV. In terms of a Hodgkin-Huxley-like model¹⁰ this corresponds to control of the channel by a single gating particle having 5.5 electronic charges associated with it. The normal channels require a multi-state model (several gating particles in Hodgkin-Huxley terms), making the conductance-voltage relation much less steep near its midpoint. The limiting steepness at very negative voltages may be similar to that for the threshold channels. Na conductance in Pronase-treated axons has been reported to change e-fold in 4.2 mV¹¹ to 5.3 mV¹². These values may require reevaluation, however, because of the contribution made by threshold channels.

Sodium channels are normally inactivated during a maintained depolarization, and threshold channels share this property. Figure 4 shows three I_{Na} tails following the specified activating pulses from an axon with its inactivation mechanism intact, that is, not Pronase-treated. After 0.3 ms at $+40$ mV or 3 ms at -40 mV, inactivation has not progressed very far, and the slow components in those traces are identical. Both are about as large as can be recorded in a fibre with the inactivation mechanism functioning. Inactivation proceeds rapidly at $+40$ mV, however, and after 3 ms the slow component is virtually absent. Initial tail amplitude was also greatly reduced. Thus, both normal and threshold channels are inactivated with a rapid time course at $+40$ mV.

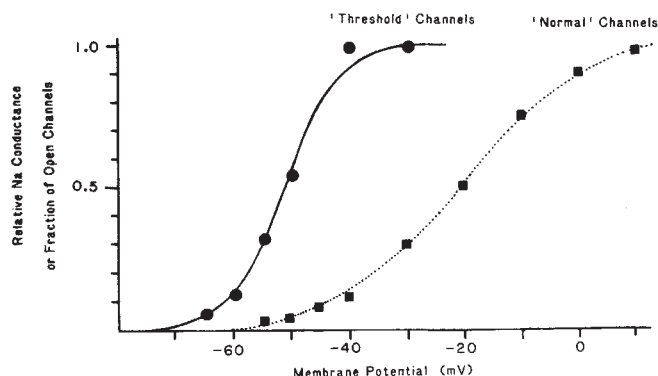


Fig. 3 Steady-state voltage dependence of Na conductance for 'threshold' versus 'normal' channels obtained in a Pronase-treated axon. Maximum conductance was 0.13 mS cm^{-2} for threshold channels as opposed to 4.3 mS cm^{-2} for normal channels, all measured in 60 mM Na . Conductance of normal channels (■) was measured at -80 mV as $\Delta I_{\text{Na}}/\Delta V$, $30 \mu\text{s}$ after termination of an activating depolarization to the indicated membrane potential¹¹. The dotted curve was drawn by eye. Conductance of threshold channels (●) was estimated from tail current amplitude (at -80 mV) 2.5 ms after termination of the activating pulse. The solid curve represents a Boltzman distribution between open and closed channels such that the fraction of open channels = $e^{(V-V_{1/2})Z/25}/[1 + e^{(V-V_{1/2})Z/25}]$ where V is membrane voltage (in mV), $V_{1/2} = -51 \text{ mV}$, and $Z = 5.5$ electronic charges moving entirely across the membrane's electric field. Same axon as in Fig. 1; 60 mM Na , 200 mM TMA .

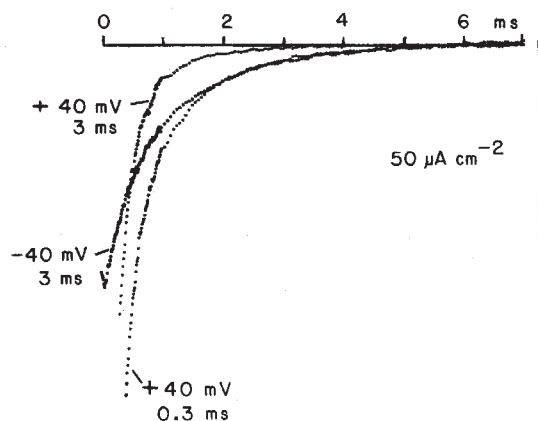


Fig. 4 Tail currents from a non-Pronase-treated axon with its inactivation mechanism intact. The slow component of current decay is identical following both the small pulse (-40 mV , 3 ms) and the brief, large pulse ($+40 \text{ mV}$, 0.3 ms), neither of which produced significant inactivation. The slow component is nearly eliminated following the longer pulse to $+40 \text{ mV}$ (3 ms) which also produced substantial inactivation of the fast component. The $+40 \text{ mV}$ traces are truncated; initial amplitudes were $670 \mu\text{A cm}^{-2}$ (0.3 ms) and $320 \mu\text{A cm}^{-2}$ (3 ms). JN203G; 120 mM Na , 200 mM TMA .

Sodium channels similar to threshold channels have not been described for other preparations in studies using either conventional or single-channel techniques. Single-channel measurements should be a sensitive way of independently discerning these unusual channels. To keep the number of open Na channels conveniently small, these measurements are often made with small depolarizations, which would selectively activate threshold channels, at least in squid axon. The slow closing kinetics we report here as being diagnostic of these channels would be manifest in single-channel data as events with unusually long open times that were particularly evident between -70 and -40 mV . Unfortunately, single-channel data are not available for squid axon.

Thus, our results reveal the existence of a small but distinct second population of Na channels, the threshold channels. Compared with the normal channels, the threshold channels activate at relatively negative voltages and close very slowly once opened. Triggering the action potential is the probable function of the threshold channels. The normal Na channels, activating over a more positive voltage range, supply the large current required for rapid conduction. One advantage in this division of labour is noise reduction: there are fewer channels operating in the threshold region, and therefore less chance of a spurious action potential being triggered by the random, spontaneous opening of enough channels to reach threshold. The overall importance of threshold-type channels as described here is unknown. They must be important in squid axon for phenomena like repetitive firing. If similar channels exist in different regions of individual neurones, for example, dendrites or axon hillocks, they should have profound effects on determining the site of impulse initiation^{6,13,14}, and if they exist in autorhythmic cells, would greatly influence pacemaking or bursting behaviour.

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- Hagiwara, S. *Membrane Potential-dependent Ion Channels in Cell Membranes: Phylogenetic and Developmental Approaches*, 118 (Raven, New York, 1983).
- Thompson, S. H. & Aldrich, R. W. in *The Cell Surface and Neuronal Function* (eds Cotman, C. W., Poste, G. & Nicolson, G. L.), 49–85 (Elsevier, Amsterdam, 1980).
- Dubois, J. M. *J. Physiol., Lond.* **318**, 297–316 (1981).
- Pappone, P. A. *J. Physiol., Lond.* **306**, 377–410 (1980).
- Ten Eick, R., Yeh, J. & Matsuki, N. *Biophys. J.* **45**, 70–73 (1984).
- Llinas, R. & Sugimori, M. *J. Physiol., Lond.* **305**, 197–213 (1980).
- Matteson, D. R. & Armstrong, C. M. *J. gen. Physiol.* **79**, 739–758 (1982).
- Armstrong, C. M. & Bezanilla, F. *J. gen. Physiol.* **63**, 533–552 (1974).
- Armstrong, C. M. & Gilly, W. F. *J. gen. Physiol.* **74**, 691–711 (1979).
- Hodgkin, A. L. & Huxley, A. F. *J. Physiol., Lond.* **117**, 500–544 (1952).
- Gilly, W. F. & Armstrong, C. M. *J. gen. Physiol.* **79**, 935–964 (1983).
- Oxford, G. S. *J. gen. Physiol.* **77**, 1–22 (1981).
- Moore, J. W. & Westerfield, M. *J. Physiol., Lond.* **336**, 285–300 (1983).
- Moore, J. W., Stockbridge, N. & Westerfield, M. *J. Physiol., Lond.* **336**, 301–311 (1983).

Current generated by backward-running electrogenic Na pump in squid giant axons

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The sodium pump of animal cells is electrogenic^{1,2}, that is, it normally exports more sodium ions than it imports potassium ions³. In the squid giant axon, the resulting net outward electric current⁴ has a density of a few $\mu\text{A cm}^{-2}$, and contributes 1–2 mV to the resting membrane potential^{5,6}. The pump is driven by the free energy of hydrolysis of ATP, and in some instances^{7–10} it has been possible to run the pump backwards and synthesize ATP by lowering the $[\text{ATP}]/[\text{ADP}] \cdot [\text{P}_i]$ ratio and steepening the transmembrane Na^+ and K^+ gradients. Here we have examined the question of whether a backward-running sodium pump conserves its $\text{Na}^+/\text{K}^+ > 1$ stoichiometry. We demonstrate reversal of the sodium pump of squid giant axon, and find that backward pumping indeed produces a net inward electric current. This current is voltage-sensitive. Our observations have mechanistic implications for models of the sodium pump.

It is generally accepted that the sodium pump normally operates with $\text{Na}^+/\text{K}^+ > 1$ stoichiometry. What is less clear is whether this stoichiometry is fixed or variable and dependent on conditions such as steepness of the ion gradients, membrane potential and available free energy of ATP hydrolysis². One extreme situation is a reversal of the pump's operation, resulting in synthesis of ATP. If the pump's normal stoichiometry (say, $3\text{Na}^+/2\text{K}^+$) were conserved, one should find an inward pump current. We have tested this hypothesis by forcing the sodium pump of squid giant axon to run backward, and simultaneously

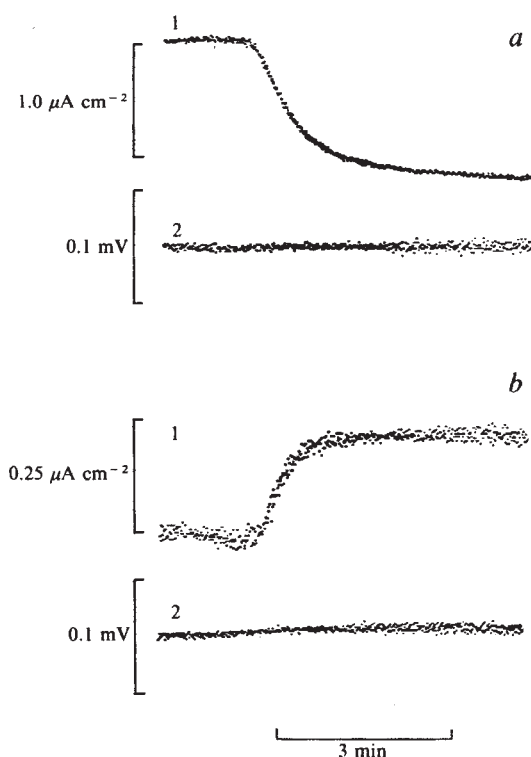


Fig. 1 Electrogenic pump measurements in squid giant axons. *a*, Axon bathed in artificial seawater containing (in mM) 425 NaCl, 10 KCl, 10 CaCl₂, 25 MgCl₂, 25 MgSO₄, 5 Tris-HEPES pH ~7.7 and 2×10^{-7} M tetrodotoxin, and internally dialysed with a solution containing (in mM) 50 NaCl, 200 K-glutamate, 100 K-HEPES pH 7.4, 250 glycine, 10 MgCl₂, 1 EGTA, 5 ATP, 3 phosphoarginine (PA) and 3 phosphoenolpyruvate (PEP), the latter four as potassium salts. 1, Holding current. About 1.3 min into the record, 10^{-5} M H₂DTG was added to the seawater. The resulting change in holding current density was $-1.1 \mu\text{A cm}^{-2}$. 2, Membrane potential. Holding potential varied less than 20 μV during the response. Temperature 17 °C. *b*, Axon bathed in K⁺-free artificial seawater (NaCl replaced KCl). Internal fluid was Na⁺-free (equimolar replacement with *N*-methyl-D-glucamine) and contained no ATP, PA or PEP, but 5 mM each of ADP and P_i, as well as 1 mM diadenosine pentaphosphate (an inhibitor of adenylate kinase) and 50 μM atractyloside (an inhibitor of mitochondrial ATP/ADP transfer). 1, Holding current; 2, membrane potential. The holding current change on addition of 10^{-5} M H₂DTG to the seawater was $0.23 \mu\text{A cm}^{-2}$. The more rapid action of H₂DTG (compared with the forward case) can be explained by the absence of potassium from the seawater. Temperature 17 °C.

Methods: The axons were mounted in a Lucite chamber $9 \times 3 \times 3$ mm, one side of which was a blackened platinum plate; the chamber was sealed at both ends with petroleum jelly. Artificial seawater superfused the axon at about 1 ml min^{-1} . A cellulose acetate capillary (diameter 140 μm ; porous in its middle) was threaded through the axon, and a conventional 'piggy-back' voltage-clamp electrode assembly, made of a 100–120 μm , 0.5 M KCl-filled glass microelectrode with a 75 μm blackened platinum wire glued to it, was placed alongside the capillary and centred in the chamber. The current-passing platinum wire protruded 1–2 mm beyond the end of the chamber. The flow rate of the internal dialysis fluid was $\sim 1 \mu\text{l min}^{-1}$. A glass capillary electrode, similar to the internal one, was dipped into the chamber. Both electrodes were connected, via matched calomel electrodes, to a 'slow' (time constant 0.1 s) and very stable voltage-clamp device. When a steady perfusion regime had been achieved, the axon was voltage-clamped, usually at -60 mV or at its resting potential. Both holding potential and clamp current were digitized, collected and stored by a microcomputer. Ten data points were sampled and averaged every 825 ms for each point displayed in the figure. Outward holding current is displayed upward. To measure the magnitude of the change in holding current, straight lines were fitted by least-squares analysis to the linear portions of the record before and after the transient, and the vertical distance between the two lines was evaluated at the midpoint of the transient.

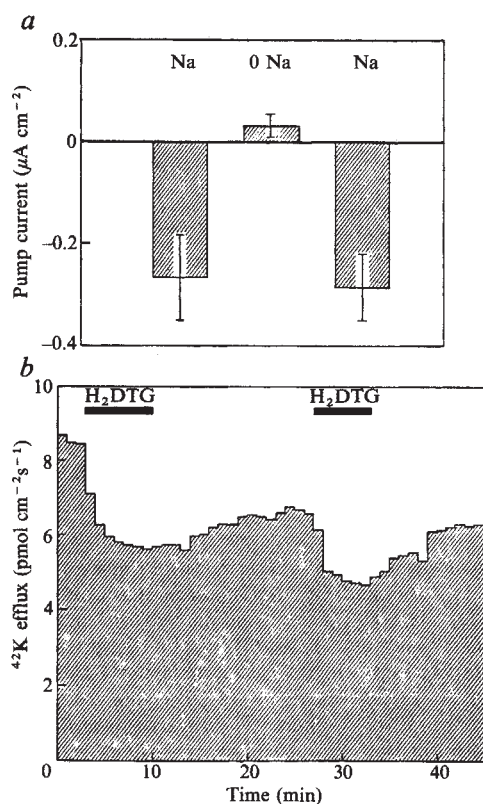


Fig. 2 Electrogenic current and potassium efflux during reverse pumping. *a*, Average ($\pm$ s.e.m.) pump current in the inward direction (combined results of six tests on five axons) before (-0.27 ± 0.08), during ($+0.03 \pm 0.02$) and after ($-0.29 \pm 0.06 \mu\text{A cm}^{-2}$) removal of sodium from the external seawater (replacement, *N*-methyl-D-glucamine). Bars show s.e.m. Sodium-free internal fluid throughout, as in Fig. 1*b*. Temperature 17 °C. *b*, Inhibition of ^{42}K efflux by H₂DTG. The effect of adding 10^{-5} M H₂DTG on ^{42}K efflux is shown for an axon held at -60 mV . Solutions as in Fig. 1*b*. The time during which H₂DTG was present in the external solution is indicated by the horizontal bar. A second application and washout of H₂DTG is also shown. The magnitude of the first response was $3.4 \text{ pmol cm}^{-2} \text{ s}^{-1}$, and of the second, $2.0 \text{ pmol cm}^{-2} \text{ s}^{-1}$. Temperature, 17 °C.

measuring cardiotoxic steroid-sensitive membrane current under voltage-clamp control.

Giant axons from the hindmost stellar nerve of the squid, *Loligo pealei*, were cleaned, mounted in a horizontal chamber and superfused with artificial seawater at either 17 or 24 °C. The axons' intracellular composition was controlled by internal dialysis using a porous capillary¹¹. Membrane potential was controlled by a slow and very stable voltage-clamp apparatus (R.F.R. *et al.*, in preparation). The principle of the pump current measurement¹² is as follows: sudden exposure of the voltage-clamped cell to a cardiotoxic steroid (ouabain or the more reversible⁴ dihydrodigitoxigenin, H₂DTG) will cause a change in clamp holding current exactly equivalent to the current generated previously by the sodium pump, provided that the steroid does not alter the passive membrane conductance. This requirement has been tested and is met (P. De W. *et al.*, in preparation).

Figure 1*a* shows a representative forward pump-clamp experiment, performed on an axon superfused with 425 mM Na⁺, 10 mM K⁺ seawater and internally dialysed with a solution containing 50 mM Na⁺, 290 mM K⁺ and 5 mM ATP. Addition of 10^{-5} M H₂DTG to the seawater produced an inward change in holding current ($1.1 \mu\text{A cm}^{-2}$). (For comparison, typical peak sodium channel membrane current densities in squid axon are $\sim 2\text{--}5 \text{ mA cm}^{-2}$, and peak gating current densities, $\sim 30 \mu\text{A cm}^{-2}$.) The axon in Fig. 1*b*, on the other hand, was bathed in K⁺-free seawater and internally dialysed with Na⁺-

Table 1 Cardiotonic steroid-sensitive currents and fluxes in squid axons measured in conditions designed to produce reverse sodium pumping

Measurement	Mean $\pm$ s.e.m.	Membrane potential (mV)	No. of expts	No. of axons	Remarks*
Current ($\mu\text{A cm}^{-2}$)	-0.332 ± 0.037	-65.8 ± 1.7	19	9	17 °C; 1982
	-0.145 ± 0.014	-58.2 ± 1.5	32	12	17 °C; 1983
	-0.316 ± 0.058	-53.1 ± 1.5	11	4	24 °C; 1983
Sodium influx ($\text{pmol cm}^{-2} \text{s}^{-1}$)	6.38 ± 0.70	-55.5 ± 9.5	2	2	17 °C; 1982/83
Potassium efflux ($\text{pmol cm}^{-2} \text{s}^{-1}$)	2.50 ± 0.25	-57.4 ± 1.2	12	6	17 °C; 1983

All current measurements were carried out as described in Fig. 1 legend. The seawater was K^+ -free as described for Fig. 1b; the cardiotonic steroid was either 10^{-5} M H_2DTG (reversible) or 10^{-5} M ouabain (irreversible). The internal dialysis fluid composition was as in Fig. 1b, except that $[\text{ADP}]$ and $[\text{P}_i]$ varied between 1 and 5 mM, with no obvious repercussions, and diadenosine pentaphosphate levels varied between 0.25 and 1 mM. Flux measurements were made in identical conditions except that either the seawater contained ^{22}Na or the internal dialysis fluid contained ^{42}K . Corresponding dialysis fluid or superfusate samples were collected and counted. Most of the ^{42}K experiments were conducted in the presence of 1 mM 3,4-diaminopyridine, which blocks potassium channels¹⁹.

* For unknown reasons, perhaps related to ocean temperatures or other environmental factors, the 1982 animals appeared to have higher reverse sodium pump activity than the 1983 specimens. The forward pumping rate was also higher in the 1982 animals. A comparable year-to-year variation has previously been seen in the case of Mg^{2+} extrusion by these cells²⁰.

free fluid containing 5 mM ADP, 5 mM P_i and no ATP (ATP formation was blocked by diadenosine pentaphosphate and atractyloside). Here H_2DTG caused an outward change in holding current ($0.23 \mu\text{A cm}^{-2}$), signifying the disappearance of an inward pump current. Table 1, summarizing 60 reverse pump current measurements obtained on 30 axons, suggests a considerable temperature coefficient for reverse pumping ($Q_{10} \approx 3$). The presumption that Fig. 1b truly represents inhibition of a reverse electrogenic sodium pump (and not of, say, a forward pump with $\text{K}^+ > \text{Na}^+$ stoichiometry) was corroborated in three ways. First, a ouabain-sensitive sodium influx was seen in axons dialysed with Na^+ -free fluid containing ADP and P_i (Table 1). Pump-mediated Na^+ influx into high-ADP cells is well known, but only as part of electroneutral $\text{Na}^+:\text{Na}^+$ exchange^{4,12,13}, which obviously requires intracellular Na^+ . Second, the putative inward pump current is abolished by removal of Na^+ from the seawater (Fig. 2a). Third, we measured a cardiotonic steroid-sensitive ^{42}K efflux (Table 1, Fig. 2b) into

K^+ -free seawater, a condition which precludes $\text{K}^+:\text{K}^+$ exchange, a known mode of operation of the sodium pump^{14,15}. (In unclamped axons engaged in forward pumping, ouabain causes ^{42}K efflux to increase through activation of K^+ channels following the depolarization which results from inhibition of the electrogenic sodium pump⁶). Simultaneous ^{42}K efflux and inward pump current measurements were made in 11 instances on 7 axons. The average $(\text{K}^+ \text{ flux})/(\text{net current})$ ratio was 2.7 ± 0.5 , or one elementary charge carried in for every 2–3 K^+ ions pumped out. This suggests a Na^+/K^+ stoichiometry of 3/2 or 4/3.

A remarkable feature emerges when reverse electrogenic pump current is plotted against membrane potential (Fig. 3). In five cases from four axons, pump current measurements at a given potential were combined with measurements at another potential, and without exception, revealed a strong voltage dependence. Interestingly, the voltage dependence represents a negative pump conductance: contrary to simple thermodynamic expectation, the inward current decreases as the cell is made more negative. This may mean either that the pump operates in series with a voltage-sensitive gate¹⁶ or that a rate-limiting kinetic step in the pump cycle is inhibited by negative membrane potentials^{17,18}.

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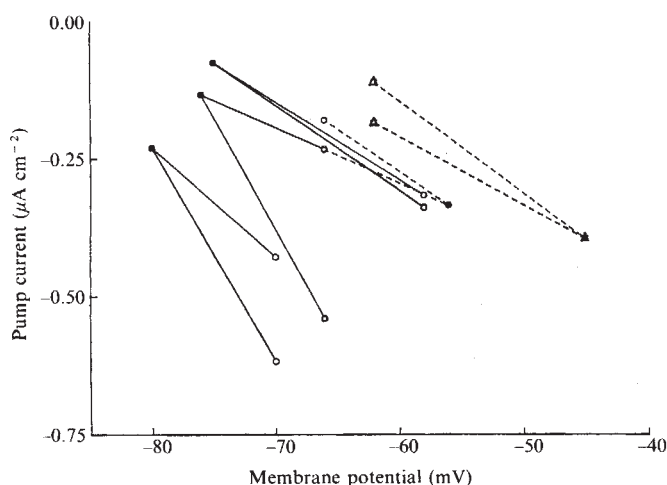


Fig. 3 Relationship between reverse pump current and membrane potential. Measurements were made as described in Fig. 1 legend. All axons were exposed to K^+ -free seawater and perfused as described for Fig. 1b. Circles, 1982 data; triangles, 1983 data. The solid and dashed lines connect sets of bracketing (open symbols) and bracketed (filled symbols) measurements. Solid lines, bracketed test at hyperpolarized potential; dashed lines, bracketed test at depolarized potential. At each control voltage 10^{-5} M H_2DTG was applied, then washed out; the holding potential was changed to the test voltage during the recovery, and the axon re-exposed to H_2DTG at the test voltage; finally, during recovery from the second H_2DTG application the holding potential was changed back to the original control voltage, and the axon was exposed to H_2DTG for the third time. Temperature, 17 °C.

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1. Thomas, R. C. *Physiol. Rev.* **52**, 563–594 (1972).
2. De Weer, P. in *MTP International Review of Science, Physiology Ser. 1*, Vol. 3 (ed. Hunt, C. C.) 231–278 (Butterworths, London 1975).
3. Sen, A. K. & Post, R. L. *J. biol. Chem.* **239**, 345–352 (1964).
4. Abercrombie, R. F. & De Weer, P. *Am. J. Physiol.* **235**, C63–C68 (1978).
5. De Weer, P. & Geduldig, D. *Science* **179**, 1326–1328 (1973).
6. De Weer, P. & Geduldig, D. *Am. J. Physiol.* **235**, C55–C62 (1978).
7. Garrahan, P. J. & Glynn, I. M. *J. Physiol., Lond.* **192**, 237–256 (1967).
8. Lant, A. F. & Whittam, R. J. *Physiol., Lond.* **199**, 457–484 (1968).
9. Lew, V. L., Glynn, I. M. & Ellory, J. C. *Nature* **225**, 865–866 (1970).
10. Chmouliovsky, M. & Straub, R. W. *Pflügers Arch. ges. Physiol.* **350**, 309–320 (1974).
11. Brinley, F. J. Jr & Mullins, L. J. *J. gen. Physiol.* **50**, 2303–2331 (1967).
12. De Weer, P. *Ann. N.Y. Acad. Sci.* **242**, 434–444 (1974).
13. Glynn, I. M. & Hoffman, J. F. *J. Physiol., Lond.* **218**, 239–256 (1971).
14. Glynn, I. M., Lew V. L. & Luthi, V. J. *Physiol., Lond.* **207**, 371–391 (1970).
15. Simons, T. J. B. *J. Physiol., Lond.* **237**, 123–155 (1974).
16. De Weer, P. in *Electrogenic Transport: Fundamental Principles and Physiological Implications* (eds Blaustein, M. P. & Lieberman, M.) 1–15 (Raven, New York, 1984).
17. Gradmann, D., Hansen, U.-P. & Slayman, C. L. *Curr. Topics Membrane Transport* **16**, 257–276 (1982).
18. Glynn, I. M. in *Electrogenic Transport: Fundamental Principles and Physiological Implications* (eds Blaustein, M. P. & Lieberman, M.) 33–48 (Raven, New York, 1984).
19. Yeh, J. Z., Oxford, G. S., Wu, C. H. & Narahashi, T. *J. gen. Physiol.* **68**, 519–535 (1976).
20. De Weer, P. *J. gen. Physiol.* **68**, 159–178 (1976).

Mechanism of ion permeation through calcium channels

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Calcium channels carry out vital functions in a wide variety of excitable cells¹⁻⁴ but they also face special challenges. In the medium outside the channel, Ca^{2+} ions are vastly outnumbered by other ions. Thus, the calcium channel must be extremely selective if it is to allow Ca^{2+} influx rather than a general cation influx. In fact, calcium channels show a much greater selectivity for Ca^{2+} than sodium channels do for Na^+ (refs 5-9) despite the high flux that open Ca channels can support^{3,4}. Relatively little is known about the mechanism of ion permeation through Ca channels. Earlier models assumed ion independence⁵ or single-ion occupancy¹⁰⁻¹⁶. Here we present evidence for a novel hypothesis of ion movement through Ca channels, based on measurements of Ca channel activity at the level of single cells or single channels. Our results indicate that under physiological conditions, the channel is occupied almost continually by one or more Ca^{2+} ions which, by electrostatic repulsion, guard the channel against permeation by other ions. On the other hand, repulsion between Ca^{2+} ions allows high throughput rates and tends to prevent saturation with calcium.

Single ventricular cells were obtained from adult guinea pigs by enzymatic dissociation¹⁷ and a suction pipette was used for voltage clamp and internal dialysis¹⁸. To minimize current flow through channels other than the Ca channel, internal and external solutions were kept Na^+ - and K^+ -free⁶.

Figure 1a shows how the Ca current (I_{Ca}) varies with the bathing calcium concentration ($[\text{Ca}]_0$). The peak magnitude of I_{Ca} increases almost proportionally with $[\text{Ca}]_0$ over the range 1-10 mM, with increasing saturation as $[\text{Ca}]_0$ approaches 30 mM. This type of $[\text{Ca}]_0$ dependence is similar to that found for Ca channels in cardiac preparations and other excitable cells^{1,2,11-16}. The saturation of I_{Ca} has been attributed to a single divalent cation-binding site within the Ca channel with a dissociation constant for Ca^{2+} (K_{mCa}) of ~10 mM (refs 11-16).

The interaction between Ca^{2+} and the channel can also be gauged by using Ca^{2+} to block the movement of other ions through the Ca channel. As Fig. 1b illustrates, addition of 0.6 mM Ca_0 to the bathing medium reduced the inward current in the presence of 10 mM Ba to less than half; in four cells the average decrease of current was to $41 \pm 4\%$ of that in the absence of Ca. The Ca-sensitivity of Ba currents is similar to the Ca-sensitivity of Sr action potentials in cardiac Purkinje fibres¹⁰.

Figure 1c shows that Ca channel current carried by Na^+ (150 mM) is even more sensitive to $[\text{Ca}]_0$. Addition of only 1.3 μM Ca to the bathing medium lowered the Na influx through the channel to $33 \pm 4\%$ in three cells. This result is consistent with previous work in heart¹⁹, snail neurones¹⁴, skeletal muscle²⁰ and other excitable cells¹³.

The very different estimates of K_{mCa} in Fig. 1a-c are inconsistent with competition for a single binding site. The discrepancy between various estimates of K_{mCa} would be even greater if allowance were made for changes in surface potential and binding of other ions. The estimate of K_{mCa} from Fig. 1a would be revised upward if allowance were made for effects of surface potential on the degree of Ca channel opening. On the other hand, estimates of K_{mCa} from Fig. 1b and c would have to be revised downward if binding of Ba or Na were taken into account.

The extreme $[\text{Ca}]_0$ -sensitivity of Na movements through the Ca channels has been attributed to a high-affinity, regulatory Ca-binding site outside the channel, distinct from a relatively low-affinity Ca-binding site that lies within the channel and controls ion permeation¹⁶. Figure 2 shows another test of the one-site model which leads us to a different interpretation of

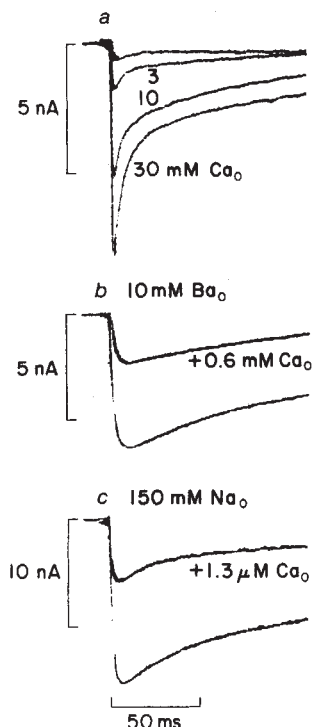


Fig. 1 Comparison of the ability of extracellular Ca to act as a charge carrier through the Ca channel (a) with its ability to block current through the channel carried by other ions (b, c). All records show net ionic currents after subtraction of linear leak and capacity currents determined from hyperpolarizing pulses. a, Currents recorded in consecutive runs with 1, 3, 10 and 30 mM $[\text{Ca}]_0$. Depolarizing pulses to +20 mV from a holding potential of -40 mV. External and internal solutions were Na^+ - and K^+ -free. Cell 165C. The internal solution contained (in mM): 75.5 Cs-aspartate, 75.5 Cs-phosphate, 10 Cs-EGTA, 6 HEPES (pH 7.4). The external solution contained (in mM): 85 Cs-aspartate, 146 sucrose, 2.8 MgCl_2 , 5 glucose, 5 HEPES (pH 7.4) and CaCl_2 or BaCl_2 as noted in the figures. Series resistance compensation was used to offset the ohmic drop resulting from current flow through the tip of the suction pipette. Current signals were recorded and analysed on a PDP 11-03 laboratory computer. All experiments were done at room temperature (21 °C). b, Block of Ba current through Ca channels by addition of external Ca. Records shown were obtained with 10 mM $[\text{Ba}]_0$ in the absence and presence of 0.6 mM $[\text{Ca}]_0$. Voltage clamp pulse from -40 mV to +20 mV. Cell 180N. c, Block of Na current through Ca channels by addition of external Ca. The control record was obtained with the standard internal solution (see text) and the following external solution: 150 mM NaCl; 2 mM EGTA, 12.5 μM tetrodotoxin (TTX), 10 mM HEPES (pH 7.6). $[\text{Ca}]_0$ was then increased to 1.3 μM by changing to the following solution: 150 mM NaCl, 2 mM total HEDTA, 1 mM total Ca, 12.5 μM TTX, 10 mM HEPES (pH 7.6, apparent stability constant for CaHEDTA taken as $7.6 \times 10^5 \text{ M}^{-1}$). Pulse from -60 mV to +10 mV. Cell 207H.

the various effects of Ca^{2+} : a shows the behaviour expected for a simple one-site channel. In mixtures of Ca and Ba in which $[\text{Ca}]_0 + [\text{Ba}]_0$ is held constant at 10 mM, the total current can never be smaller than that in either 10 mM $[\text{Ca}]_0$ or $[\text{Ba}]_0$ alone; the total current must be a monotonic function of the mole fraction. This prediction was tested experimentally with recordings of whole-cell current in guinea pig ventricular myocytes (Fig. 2b). In contrast to the properties expected for a simple one-site channel, the measured current is not a monotonic function of the mole fraction, $[\text{Ba}]_0/([\text{Ba}]_0 + [\text{Ca}]_0)$, but goes through a clear minimum as 70% of the external Ca is replaced by Ba. Similar behaviour of currents in mixtures of permeant ions has been reported previously for gramicidin channels^{21,22}, K channels²³ and Cl channels^{24,25}. Such 'anomalous mole fraction effects'²⁶ have been accounted for by models which include two (or more) ion-binding sites within the channel and repulsive

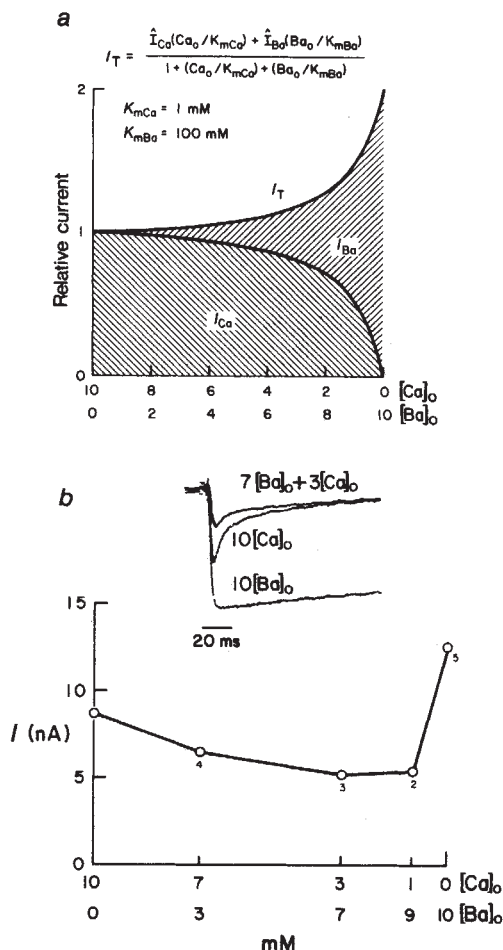


Fig. 2 The mole-fraction dependence of Ca channel current predicted for a channel with one ion-binding site (*a*) compared with that found experimentally (*b*). *a*, Computed values of total current (I_T) and its components I_{Ca} and I_{Ba} , calculated for a one-site channel using the equation shown. The dissociation constants of the site for Ca and Ba (K_{mCa} and K_{mBa} respectively) were chosen as shown in the inset. The maximum Ba current (I_{Ba}) was made twice as large as the maximum Ca current (I_{Ca}) because Ba currents are larger than Ca currents in these cells. No matter what values are chosen for K_{mCa} , K_{mBa} , I_{Ca} and I_{Ba} , the one-site model always predicts that I_T is a monotonic function of the mole fraction. *b*, Peak amplitude of leak-subtracted inward current plotted against mole fraction. Voltage clamp pulse from -40 mV to $+10$ mV. To ensure that the anomalous mole-fraction dependence was not the result of rundown, the solutions were applied in the order indicated by the numbers next to each data point. Individual current records are shown in the inset. Cell 166A.

interactions between ions in the doubly occupied state^{27,28}.

The main uncertainty concerning the results in Fig. 2 is that changes in the peak current may not reflect changes in the current through open channels (i) but rather changes in the probability of channels being open (p). To measure single-channel current, we performed ensemble fluctuation analysis²⁹ on whole-cell recordings³⁰ from single frog ventricular cells³¹. Figure 3 shows a determination of i during the inactivation phase of Ca channel current. Estimates of i , the number of functional channels (N_F), and the peak probability of channel opening (p_{max}) are in reasonably good agreement with results of ensemble fluctuation analysis during the rising phase of Ca channel current³¹. As Fig. 3c shows, the main point here is that with $[Ca]_0 = 3$ mM and $[Ba]_0 = 7$ mM, i is significantly smaller than with $[Ca]_0 = 10$ mM or $[Ba]_0 = 10$ mM. Similar results were obtained in rat ventricular cells at a test potential of $+20$ mV (B. P. Bean and M. C. Nowicky, unpublished).

To explore the implications of the anomalous mole-fraction effect, we modelled the Ca channel as a single-file pore with

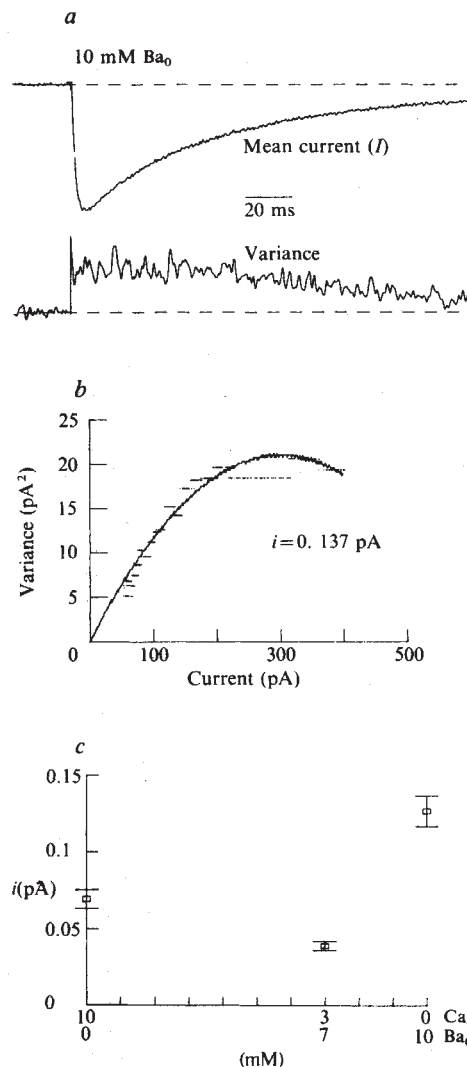
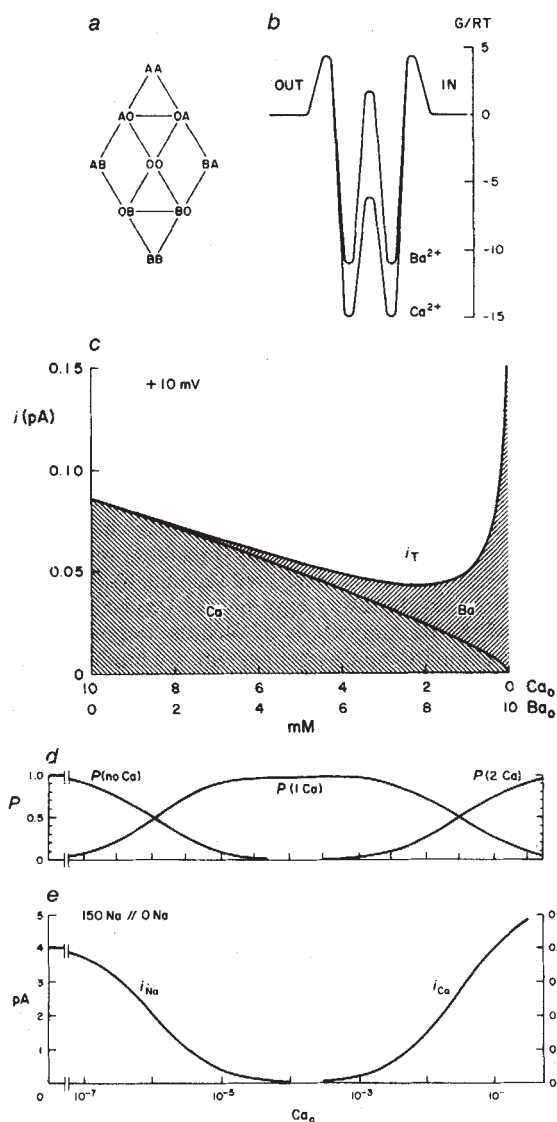


Fig. 3 The anomalous mole-fraction effect is a property of the open Ca channel. Single-channel currents were estimated by non-stationary fluctuation analysis²⁴ as illustrated in *a* and *b*. Whole-cell currents were recorded from single frog ventricular cells using the patch-clamp technique²⁵. Fifty consecutive current records obtained with voltage steps from -60 mV to $+10$ mV were lined up in time and the mean and variance were calculated for each sampling point. The time course of the mean current (after subtraction of linear leak and capacity currents) and the variance are shown in *a*. The variance associated with the holding current was taken as the baseline (dashed line). *b*, Plot of the variance associated with the falling phase of the current as a function of the mean current. The data are fitted to the equation: $\text{variance} = iI - I^2/N_F$, where $I = N_F iP$. These equations describe the behaviour of N_F independent channels with a single-channel current i and a probability P of being open²⁹. For the example shown, the best-fit parameters were $i = 0.137$ pA, $N_F = 4,340$ channels and $P_{max} = 0.66$. The external solution contained (in mM): $BaCl_2$ 10, tetraethylammonium-Cl 135, $MgCl_2$ 1, HEPES 10 (pH 7.6). The patch pipette contained (in mM): CsCl 120, $MgCl_2$ 5, EGTA 10. Cell P11B. *c*, Mean ($\pm$ s.e.m.) single-channel currents at a test potential of $+10$ mV determined by the method described above in 10 mM $[Ca]_0$ ($n = 7$ cells), 7 mM $[Ba]_0 + 3$ mM $[Ca]_0$ ($n = 6$) and 10 mM $[Ba]_0$ ($n = 7$).

two ion-binding sites (energy wells) and three energy barriers (for example, see refs 27, 28, 32). Figure 4a shows the various states of ion occupancy for the case of two permeant cations (for example, Ca and Ba). Rates of transition between connected states were calculated from Eyring rate theory, with energy profiles for Ca and Ba like those shown in Fig. 4b. For simplicity, the ion-binding sites (energy wells) were assumed to be equally spaced within the membrane; symmetry of energy profiles was

Fig. 4 Two-binding site model for the calcium channel. *a*, State diagram for the case of two ions. A and B, present on both sides of the membrane. An empty site is denoted by O. As the diagram indicates, single-filing is assumed. *b*, Free energy profiles for a single ion (Ca^{2+} or Ba^{2+}) in the absence of both an applied electric field and other ions within the channel. To reduce the number of adjustable parameters, barriers and wells are assumed to be spaced equally, $1/6$ th of the membrane thickness apart; chemical energy profiles are assumed to be symmetrical with respect to the centre of the membrane. As in earlier treatments of this type (see ref. 28 for earlier references), rates of transition were calculated by Eyring rate theory expressions of the form $\text{rate} = Q \exp(-\Delta G)$ (for entry rates this expression is multiplied by the ion activity in the medium from which the ion enters the channel). By convention, $Q = (kT h^{-1})$ as in treatments of ion permeation through Na and K channels^{28,32,34}. The free energy change in surmounting a barrier ΔG is taken as the sum of three terms: a purely chemical ΔG^0 (as indicated in *b*), an electrical term $\pm z_i V/6$, and (when the far site is occupied) a repulsion term, $\pm z_i z_j \ln(F_{mm})$, where $F_{mm} = 3$ (z = valence and V = membrane potential). The choice of signs was determined by the direction of the transition relative to the applied electric field and to the position of the repelling ion. *c*, Calculated anomalous mole-fraction effect with $[\text{Cs}]_0 = 85 \text{ mM}$ and $[\text{Cs}]_i = 171 \text{ mM}$ (as in Fig. 2*b*), $[\text{Ba}]_0 = 0$ and $[\text{Ca}]_i = 0$ at $+10 \text{ mV}$. (Calculations with a computer model based on a program kindly provided by Dr T. Begenisich.) Activity coefficients for monovalents (divalents): 0.764 (0.341) outside and 0.725 (0.276) inside. Chemical component of energy profile for Cs^+ (in units of RT , where R is the gas constant and T is the absolute temperature): wells, -6 , -6 ; barriers from outside to inside, 7.8 , 10.3 , 12.8 . This profile also fits measured Ca channel reversal potentials with $[\text{Ba}]_0 = 10 \text{ mM}$ or $[\text{Ca}]_0 = 10 \text{ mM}$ and internal $\text{Cs}^{9,35}$. Ca and Ba components of net current are shown; as Cs flux is less than 1.5% of net current magnitude, it is not shown. *d*, Occupancy of the channel by Ca as a function of $[\text{Ca}]_0$. Probabilities of the channel containing 0, 1 or 2 Ca ions are shown at 0 mV in the presence of 150 mM external Na and no permeant internal ions. The chemical component of the energy profile for Na^+ (in units of RT : wells $1, 1$; barriers $6, 8, 10$ from outside to inside) also fits measured reversal potentials of $[\text{Na}]_0$ versus $[\text{Cs}]_i$ and $[\text{Ba}]_0$ versus $[\text{Na}]_i$ (unpublished results). *e*, Elementary inward currents computed for the conditions assumed in *d*. At $[\text{Ca}]_0 < 10^{-4} \text{ M}$, inward current is carried by Na (i_{Na}); Ca only carries significant current at $[\text{Ca}]_0 > 10^{-3} \text{ M}$ (i_{Ca}).



assumed in the absence of compelling reasons to the contrary. Ion-ion interactions were incorporated by a repulsion factor F which has the effect of making entry rates into a site F -fold slower and exit rates F -fold faster if the other site is occupied by another cation²⁸. For monovalent-monovalent (mm) interactions, F_{mm} was set at 3 (well within the range expected for reasonable assumptions about the dielectric environment surrounding an aqueous pore³³). For monovalent-divalent and divalent-divalent (dd) interactions, F was given by Coulomb's law without additional adjustable parameters: thus $F_{dd} = (F_{mm})^{2-2} = 3^4 = 81$. Once specific values for the barrier heights and well depths are assigned to each ion, the model allows the computation of ion fluxes and ion occupancies in the presence of three permeant ions (for example, Ca, Ba and Cs).

A central feature of the model is that both binding sites have a high affinity for Ca ions. The postulated well depth for Ca of $-15RT$ corresponds to an apparent dissociation constant of $\exp(-15) = 3 \times 10^{-7} \text{ M}$, three or four orders of magnitude lower than earlier estimates¹⁰⁻¹⁶. With the barrier heights and well depths shown in Fig. 4*b*, the model can account for the present experimental results: (1) calculated unitary currents exhibit an anomalous mole-fraction dependence (Fig. 4*c*); (2) Na movements through the channel are blocked by micromolar levels of Ca (Fig. 4*e*); while (3) Ca current increases as $[\text{Ca}]_0$ is elevated, with little saturation until $[\text{Ca}]_0$ reaches several millimolar (Fig. 4*e*). According to the model, these observations may be interpreted as follows.

The anomalous mole-fraction effect arises because the channel binds Ca^{2+} more strongly than Ba^{2+} and because Ca^{2+} and Ba^{2+} ions repel each other within the channel (see refs 27, 28). When the inner site is occupied by a Ca ion, a Ba ion entering across the outer barrier is very quickly repelled back out of its shallower

well. Thus, equimolar replacement of external Ca by external Ba makes the current smaller at first because Ba^{2+} remains relatively impermeable until $[\text{Ca}]_0$ is low.

As Ca^{2+} binds so strongly, channel occupancy by a single Ca^{2+} is enough to block Na flux through the channel, and this occurs at micromolar levels of $[\text{Ca}]_0$. On the other hand, Ca flux through the Ca channel increases hand-in-hand with the probability of two Ca^{2+} ions in the channel (Fig. 4*d, e*). Because of mutual repulsion, this probability only becomes significant at millimolar $[\text{Ca}]_0$. Once double occupancy has occurred, repulsion promotes current flow. The presence of a Ca^{2+} at the outer site repels a Ca^{2+} at the inner site, increasing its chances of leaving that site and entering the cell. In this way, channel occupancy by Ca^{2+} tends to perpetuate itself, and Ca^{2+} strongly reinforces its own permeability. Only a second Ca^{2+} stays long enough to have a significant chance of displacing a predecessor Ca^{2+} . Like Ba^{2+} , Na^+ and other ions which bind less strongly than Ca^{2+} are quickly repelled out of the channel by Ca^{2+} .

This explanation helps resolve the controversy about the ionic basis of the 'slow inward current' (I_{si}) in cardiac muscle. On one hand, I_{si} has been called $I_{\text{Ca}^{2+}, \text{Na}^+}$ because Na^+ ions can support Mn^{2+} - and verapamil-sensitive action potentials or inward currents in the absence of external Ca¹⁹. On the other hand, in the presence of external Ca, prolonged removal of external Na reduces I_{si} very little^{7,8}. Our results suggest that there is no contradiction between the channel's ability to support a large Na influx in the absence of Ca and its ability to reject Na in the presence of Ca. The high transport number for Ca in

physiological conditions justifies the simple label 'Ca channel' in the same way that the Na selectivity of tetrodotoxin (TTX)-sensitive channels justifies the name 'Na channel'.

This report is the first to argue that Ca channels are multi-ion, single-file pores, rather than one-ion pores that function by simple mutual exclusion. Our model is very different from the two-site model recently proposed by Kostyuk *et al.*¹⁶—they assumed that the pore can only contain one ion at a time and concluded that the channel selects for Ca over Ba at its outer binding site with $K_{mCa} = 10$ mM and $K_{mBa} = 100$ mM. To explain Ca^{2+} block of Na^{+} influx, they had to postulate an additional external regulatory site with high affinity for Ca which controls the structure of the pore. Their model cannot account for the anomalous mole-fraction effect and it predicts that block of Ba currents by Ca^{2+} would require a $[Ca]_0$ at least 10 times higher than that described here.

As multi-ion pores, Ca channels resemble several other channels that are selective for Na, K or Cl; however, the proposed selectivity mechanism differs from previous descriptions of other biological channels. Unlike Na channels^{32,34}, Ca channels seem to bind the preferred ion more strongly than other physiological ions (see also refs 11, 16). Ca is selected as much by deep-energy wells as by a relatively low-energy barrier ('selectivity filter'). The nature of the binding site is of obvious importance. It is interesting to compare the Ca channel with Ca binding proteins such as troponin and calmodulin, or small organic Ca buffers such as EGTA. Like the proposed Ca binding sites in our model channel, the Ca-specific coordination sites on these molecules bind Ca^{2+} with high affinity (effective $K_d < 1$ μ M), in preference to other cations (for example, Ba^{2+} , Sr^{2+} , Mg^{2+} , Na^{+}). Although detailed differences are to be expected, it seems possible that Ca channels and intracellular Ca receptors may share similar mechanisms of Ca selectivity.

After submission of this manuscript and publication of preliminary communications of this work^{35,36}, we received a manuscript with a similar theoretical treatment of Ca channels in skeletal muscle by W. Almers and E. W. McCleskey³⁷.

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Complete tyrosine-O-sulphation of gastrin in neonatal rat pancreas

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Modification of tyrosine residues of proteins has attracted great interest. Although phosphorylation has received most attention¹⁻³, tyrosine-O-sulphation is also common⁴ and has been closely investigated in the hormones gastrin and cholecystokinin (CCK). CCK regulates pancreatic enzyme secretion. It is always sulphated⁵⁻⁹ and sulphation increases its pancreatic secretagogue activity 100-fold¹⁰⁻¹³. By contrast, only half of gastrin molecules are tyrosine-O-sulphated⁵⁻⁹ and sulphation does not influence the ability of gastrin to regulate gastric acid secretion⁶. We now report that in neonatal rat pancreas, which is the major source of gastrin in the newborn rat¹⁴, gastrin is completely sulphated. Moreover, the disappearance of gastrin from the pancreas during the first weeks after birth parallels the appearance of CCK in the intestine. Since sulphation is essential for pancreatic secretagogue activity of gastrin as well as of CCK¹⁰⁻¹³, pancreatic gastrin in the neonate may be the equivalent of intestinal CCK in the adult.

The pancreas, antrum and small intestine of 0-, 4-, 7-, 14- and 21-day-old Wistar rats and of 17-day fetuses were dissected out and frozen immediately on dry ice. Tissues were extracted by boiling in water and the pellet was re-extracted in cold 0.5 M acetic acid. The concentrations of gastrin and CCK in the extracts were measured by sequence-specific radioimmunoassays (RIAs), including one which distinguishes between non-sulphated and sulphated peptides^{9,15-17}. The extracts were further analysed by Sephadex G-50 superfine chromatography and ion-exchange HPLC (see Fig. 3 legend). Both gel and ion-exchange chromatography were monitored using gastrin RIAs, one (using antiserum no. 2604) measuring sulphated and non-sulphated gastrins¹⁵ and another (using antiserum no. 2605) measuring only non-sulphated gastrins^{9,16}.

Figure 2 shows the fetal and postnatal changes in pancreatic gastrin and intestinal CCK concentrations. At birth, pancreatic gastrin concentration was high whereas gastrin and CCK concentrations in the small intestine were low, relative to adult concentrations. After the fourth postnatal day, pancreatic gastrin rapidly disappeared, concomitant with the appearance of CCK in the intestine.

Gel chromatography of the pancreatic extracts followed by RIA using the antiserum that binds both sulphated and non-sulphated gastrins revealed only one peak of immunoreactivity, in a position corresponding to 'little' gastrin (or gastrin-17), and which was not recognized by the antiserum that binds only non-sulphated gastrins. The pancreatic and antral little gastrin was further characterized by high-pressure ion-exchange chromatography of the gastrin-containing fractions from the gel chromatography. By contrast with antral gastrin which was partially sulphated, gastrin from the neonatal pancreas was completely sulphated. Gastrin immunoreactivity in the pancreas co-eluted with both sulphated antral gastrin immunoreactivity (Fig. 3a, b) and biosynthetically ³⁵S-sulphate-labelled gastrin (Fig. 3a, c). Moreover, pancreatic gastrin was desulphated by treatment with limpet arylsulphatase (Fig. 3d).

Antral little gastrin was resolved by ion-exchange chromatography into four peaks (Fig. 3b), probably representing sulphated and non-sulphated forms of gastrin-15 and -17, since rat gastrin-17 contains arginine (Fig. 1) one amino acid after the dibasic cleavage site of gastrin-17 (ref. 18). These identifications were confirmed by the observation that on ion-exchange chromatography of trypsin-treated antral gastrin, there was a single peak for each of sulphated and non-sulphated gastrin

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- Hagiwara, S. & Byerly, L. A. *Rev. Neurosci.* **4**, 69-125 (1981).
- Kostyuk, P. G. *Biochem. biophys. Acta* **650**, 128-150 (1981).
- Tsien, R. W. A. *Rev. Physiol.* **45**, 341-358 (1983).
- Reuter, H. *Nature* **301**, 569-574 (1983).
- Reuter, H. & Scholz, H. *J. Physiol., Lond.* **264**, 17-47 (1977).
- Lee, K. S. & Tsien, R. W. *Nature* **497**, 498-501 (1982).
- Isenberg, G. Z. *Naturf.* **370**, 502-512 (1982).
- Matsuda, H., Noma, A. & Irisawa, H. *Proc. int. Un. physiol. Sci.* **15**, 50 (1983).
- Lee, K. S. & Tsien, R. W. *J. Physiol., Lond.* (submitted).
- Vereceke, J. & Carmeliet, E. E. *Pflügers Arch. ges. Physiol.* **322**, 73-82 (1971).
- Hagiwara, S., Fukuda, J. & Eaton, D. C. *J. gen. Physiol.* **63**, 564-578 (1974).
- Akaike, N., Lee, K. S. & Brown, A. M. *J. gen. Physiol.* **71**, 509-531 (1978).
- Edwards, C. *Neuroscience* **7**, 1335-1366 (1981).
- Kostyuk, P. G., Mironov, S. L. & Doroshenko, P. A. *J. Membrane Biol.* **70**, 181-189 (1982).
- Ashcroft, F. M. & Stanfield, P. R. *J. Physiol., Lond.* **323**, 93-115 (1982).
- Kostyuk, P. G., Mironov, S. L. & Shuba, Ya. M. *J. Membrane Biol.* **76**, 83-93 (1983).
- Kao, R. L. *et al. Archs Biochem. Biophys.* **203**, 587-599 (1980).
- Lee, K. S., Akaike, N. & Brown, A. M. *J. Neurosci. Meth.* **2**, 51-78 (1980).
- Garnier, D., Rougier, O., Gargouil, Y. M. & Coraboeuf, E. *Pflügers Arch. ges. Physiol.* **313**, 321-342 (1969).
- Almers, W., McCleskey, E. W. & Palade, P. T. *J. Physiol., Lond.* **332**, 52-53P (1982).
- Andersen, O. S. *Abstr. 5th int. Biophys. Congr.*, 112 (1975).
- Neher, E. *Biochim. biophys. Acta* **401**, 540-544 (1975).
- Hagiwara, S., Miyazaki, S., Krasne, S. & Ciani, S. *J. gen. Physiol.* **70**, 269-281 (1977).
- Takeuchi, A. & Takeuchi, N. *J. Physiol., Lond.* **212**, 337-351 (1971).
- Hagiwara, S. & Takahashi, K. *J. Physiol., Lond.* **238**, 109-127 (1974).
- Eisenman, G., Sandblom, J. P. & Walker, J. L. *Jr Science* **155**, 965-974 (1967).
- Urban, B. W., Hladky, S. B. & Haydon, D. A. *Fedn Proc.* **37**, 2628-2632 (1978).
- Hille, B. & Schwarz, W. *J. gen. Physiol.* **72**, 409-442 (1978).
- Sigworth, F. J. *J. Physiol., Lond.* **307**, 97-129 (1980).
- Hamill, O., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch. ges. Physiol.* **391**, 85-100 (1981).
- Bean, B. P., Nowicky, M. C. & Tsien, R. W. *Nature* **307**, 371-375 (1984).
- Begenisich, T. B. & Cahalan, M. D. *J. Physiol., Lond.* **307**, 217-242 (1980).
- Levitt, D. G. *Biophys. J.* **22**, 209-219 (1978).
- Hille, B. *J. gen. Physiol.* **66**, 535-560 (1975).
- Hess, P. & Tsien, R. W. *Soc. Neurosci. Abstr.* **9**, 509 (1983).
- Tsien, R. W., Bean, B. P., Hess, P. & Nowicky, M. C. *Cold Spring Harb. Symp. quart. Biol.* **48**, 201-212 (1983).
- Almers, W. & McCleskey, E. W. *J. Physiol., Lond.* (in the press).

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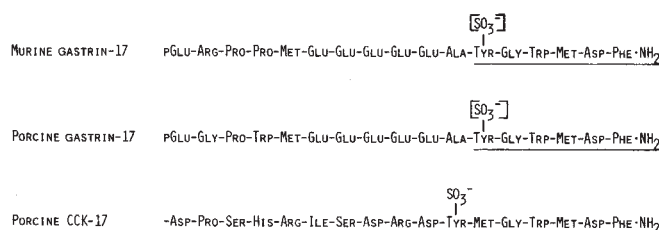


Fig. 1 The primary structure of heptadecapeptide gastrin from rat¹⁸, pig²⁷ and of the C-terminal heptadecapeptide fragment of pig CCK⁵. The heptadecapeptide amide is the principal hormonal form of gastrin. The homologous C-terminal sequences containing the biologically active sites of both gastrin and CCK are underlined. The sulpho-tyrosine groups of the gastrins are given in brackets, since only half of the gastrin-17 molecules in rat and pig antral mucosa are sulphated, in contrast to the completely sulphated porcine CCK.

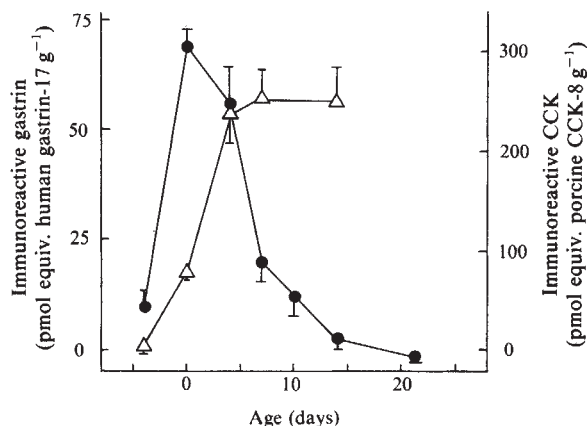


Fig. 2 The neonatal changes of gastrin in the pancreas (●) and CCK in the small intestine (Δ) of the rat. The number of rats for each point is 20 pooled in five groups of 4. Gastrin was measured in aqueous extracts with a specific RIA using a rabbit antiserum (2604; ref. 15), which detects sulphated and non-sulphated gastrins with equimolar potency, but CCK negligibly ($P < 0.0006$). CCK was measured in both aqueous and acetic acid extracts of intestine in a specific CCK RIA¹⁷, using a rabbit antiserum (G-160; ref. 28) with which sulphated gastrins react at less than 0.005% and non-sulphated gastrins not at all.

(data not shown). Most of the pancreatic gastrin co-eluted with the trypsin-labile peak of little gastrin extracted from rat antrum and is thus presumably gastrin-17, although a small peak of sulphated gastrin-15 was also seen (Fig. 3a). The proximal small intestine contains moderate amounts of gastrin in the fetal, neonatal and adult rat (ref. 14 and this study). In both adult and neonate, however, this intestinal gastrin was similar to antral gastrin in being only partially sulphated.

Although the physiological significance of the transient expression of gastrin in the neonatal pancreas is not yet clear, the fact that it is completely tyrosine-*O*-sulphated suggests a CCK-like action on surrounding exocrine cells by a paracrine mode of release. Tyrosine-*O*-sulphation of CCK increases pancreatic acinar receptor binding and secretagogue potency by 100-fold^{11,12}. Although sulphated gastrin is a weaker stimulus than sulphated CCK, this would be compensated for by the higher concentrations achieved through intrapancreatic release. Birth heralds a transition from placental to alimentary nutrition, so the dramatic changes in pancreatic gastrin and intestinal CCK concentrations in the neonate (Fig. 2) would seem to be related to the start of feeding. Pancreatic gastrin levels rise just prior to birth, before food is present in the intestinal lumen. After birth, CCK appears in the intestine and pancreatic gastrin rapidly disappears as intestinal stimuli become more important in controlling pancreatic function. Pancreatic gastrin may there-

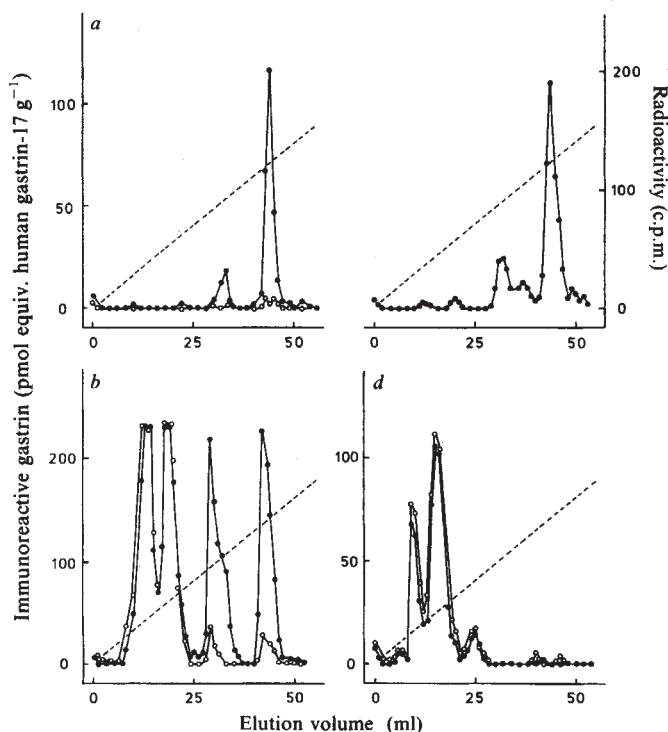


Fig. 3 *a*, Sulphation of gastrin in neonatal pancreas. Extracts were applied to a 5×50 mm Mono-Q HR5/5 anion-exchange column (Pharmacia) equilibrated with 20 mM sodium phosphate, pH 6.5. The column was washed with 10 ml 0.01% v/v acetic acid and eluted with a gradient from 0.01% to 12.5% acetic acid (----) over 50 min at room temperature with a flow of 1 ml min⁻¹ (ref. 29). One-minute fractions were collected and assayed after removing the acetic acid by freeze-drying with two gastrin RIAs using antisera 2604 (●) and 2605 (○). *b*, Sulphation of gastrin in antral extracts fractionated on Mono-Q anion-exchange column as described above. Gastrin was measured by RIAs using antisera 2604 (●) and 2605 (○) as described above. *c*, ³⁵S activity (c.p.m.; ●) from slices of rat antrum incubated with ³⁵S-sulphate in sulphate-free Hank's media for 4 h. Antral extracts were then applied to a Seppak cartridge (Waters Associates), washed extensively, eluted with methanol, dried and applied to a Sephadex G50 column. The gastrin-17-like immunoreactivity was pooled and applied to a Mono-Q column and eluted as above and ³⁵S-sulphate radioactivity counted by liquid scintillation. Incorporation of ³⁵S-sulphate activity was directly confirmed by two-dimensional thin-layer electrophoresis of alkaline hydrolysates of the peptide⁴. All the ³⁵S-sulphate radioactivity co-migrated with tyrosine-*O*-sulphate standard. *d*, Pancreatic gastrin purified by gel chromatography was treated with limpet arylsulphatase (Sigma S8629). Dried peptide was dissolved in 800 μl of 0.2 M sodium acetate, pH 5, and 250 μg arylsulphatase, dissolved in 100 μl 0.2% sodium chloride, were added and the mixture incubated at 37 °C for 2 h. The incubation mixture was diluted with water and then applied to a Mono-Q anion-exchange column, eluted as above and gastrin measured by RIA using antisera 2604 (●) and 2605 (○).

fore be important in stimulating rapid pancreatic development at birth to ensure adequate digestion when feeding commences.

In addition to stimulating pancreatic growth and secretion, gastrointestinal and pancreatic peptides also stimulate enzyme synthesis. Although this action is best understood for insulin, which selectively stimulates amylase gene transcription¹⁹, CCK-like peptides may also stimulate trypsin and chymotrypsin synthesis²⁰. These actions may be especially important in the neonate as just before parturition pancreatic enzyme concentrations rise markedly²¹. Pancreatic insulin²¹ and gastrin concentrations (this study) also show parallel rises just before parturition, suggesting that these hormones induce enzyme synthesis in the surrounding exocrine cells. Whether the actual synthesis of pancreatic gastrin including the tyrosine-*O*-sulphation, is controlled by mechanisms inherent in the gastrin-producing cell

DNA or by signals from the environment, remains to be determined.

The complete sulphation of gastrin is not restricted to the neonatal rat pancreas. The feline pancreas contains gastrin that is completely sulphated and, although the normal human pancreas is devoid of gastrin, the small intestine of the human fetus contains 30-fold more gastrin than CCK and all the gastrin molecules are sulphated. After birth, the concentration of intestinal gastrin declines and gastrin assumes the adult partly-sulphated pattern (B.N.A., in preparation). Some human pancreatic gastrinomas contain only sulphated gastrin²². In view of the localization of essentially all malignant gastrinomas to the pancreas²³ and the homology between sulphated gastrin and the transforming middle-T antigen of polyoma virus²⁴, the present results may also be of relevance to oncogenesis. Note, however, that the homology is less pronounced in the sequence corre-

sponding to the active site of gastrin, the C-terminal tetrapeptide amide.

It has previously been suggested that important changes can occur in peptide processing during neonatal development^{25,26}. Our results support the contention that an organ at different stages of development can be regulated by different peptides through modulation of a post-translational modification critical for receptor binding.

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1. Erikson, R. L., Purchio, A. F., Erikson, E., Collett, M. S. & Brugge, J. S. *J. Cell Biol.* **87**, 319–325 (1980).
2. Huttner, T. *Cell* **22**, 647–648 (1980).
3. Ushiro, H. & Cohen, S. *J. Biol. Chem.* **255**, 8353–8365 (1980).
4. Huttner, W. B. *Nature* **299**, 273–276 (1982).
5. Mutt, V. & Jorpes, J. E. *Eur. J. Biochem.* **6**, 156–162 (1969).
6. Gregory, R. A. & Tracy, H. J. *Gut* **5**, 103–117 (1964).
7. Rehfeld, J. F., Stadil, F. & Vikelsøe, J. *Gut* **15**, 102–111 (1974).
8. Rehfeld, J. F. & Larsson, L.-I. *J. Biol. Chem.* **256**, 10425–10429 (1981).
9. Andersen, B. N., Magistis, L. de & Rehfeld, J. F. *Clinica chim. Acta* **127**, 29–39 (1983).
10. Mutt, V. in *Gastrointestinal Hormones* (ed. Glass, W. B. J.) 169–221 (Raven, New York, 1980).
11. Jensen, R. T., Lemp, G. F. & Gardner, J. D. *J. Biol. Chem.* **257**, 5554–5559 (1982).
12. Dockray, G. J. *Q. J. exp. Physiol.* **58**, 163–169 (1973).
13. Jensen, S. L. *et al. Am. J. Physiol.* **238**, E186–192 (1980).
14. Larsson, L.-I., Rehfeld, J. F., Håkanson, R. & Sundler, F. *Nature* **262**, 609–610 (1976).
15. Rehfeld, J. F., Stadil, F. & Rubin, B. *Scand. J. clin. Lab. Invest.* **30**, 221–232 (1972).
16. Rehfeld, J. F., Magistis, L. de & Andersen, B. N. *Regul. Peptides* **2**, 333–342 (1981).

17. Rehfeld, J. F. *J. Biol. Chem.* **253**, 4016–4021 (1978).
18. Reeve, J. R. *et al. Peptides* **2**, 453–458 (1981).
19. Korc, M., Owerbach, D., Quinto, C. & Rutter, W. J. *Science* **213**, 351–353 (1981).
20. Solomon, T., Petersen, H., Elashoff, J. & Grossman, M. I. *Am. J. Physiol.* **236**, E714–719 (1978).
21. Pictet, R. & Rutter, W. J. in *Handbook of Physiology* Sect. 7, Vol. 1 (eds Freinkel, N. & Steiner, D. F.) 25–26 (American Physiological Society, 1972).
22. Andersen, B. N. & Stadil, F. *Regul. Peptides* **6**, 231–239 (1983).
23. Zollinger, R. M. & Coleman, D. W. *The Influence of Pancreatic Tumours on the Stomach* (Thomas, Springfield, 1974).
24. Baldwin, G. S. *FEBS Lett.* **137**, 1–5 (1982).
25. Bayon, A., Shoemaker, W. J., Bloom, F. E., Mauss, A. & Guilemin, R. *Brain Res.* **179**, 93–101 (1979).
26. Haynes, L. W., Smyth, D. G. & Zakarian, S. *Brain Res.* **232**, 115–128 (1981).
27. Gregory, H., Hardy, P. M., Jones, D. S., Kenner, G. W. & Sheppard, R. C. *Nature* **204**, 931–935 (1964).
28. Schaffmayer, A., Werner, M. & Becker, H. D. *Digestion* **24**, 146–154 (1982).
29. Baldwin, G. S., Knesel, J. & Monckton, J. M. *Nature* **301**, 435–437 (1983).

Expression and amplification of the N-myc gene in primary retinoblastoma

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Retinoblastoma, the most common intraocular tumour of childhood, probably arises from embryonal cells and occurs in hereditary and non-hereditary forms¹. Recent evidence suggests that this retinoblastoma (*Rb*) susceptibility gene located at chromosome 13q14 is actually recessive^{2–4}. Knudson has proposed that the tumour is caused by two mutational events⁵. This idea was extended by Comings⁶, who suggested that dominantly inherited tumours may result from loss or inactivation of both alleles of regulatory or suppressor genes that, when active, prevent the expression of a structural transforming gene(s) (possibly an oncogene) normally active only during embryogenesis. Despite circumstantial evidence^{3,4,7,8} for this hypothesis, no activated oncogene^{9–14} has been identified. We now report that (1) the N-myc gene is amplified 10–200-fold in two primary retinoblastomas and a retinoblastoma cell line Y79 and (2) expression of N-myc gene is highly elevated in most of the retinoblastomas examined. This finding suggests that N-myc gene may have a primary role in the tumorigenesis of retinoblastoma.

Genomic DNA was prepared from 10 primary retinoblastomas, 3 of which are described here for the first time. The other seven tumours have been karyotyped and reported previously⁸ (Table 1). Various oncogenes were used as probes in the Southern genomic blotting analysis^{15,16}. We found that two tumours (LA-RB70 and LA-RB63-1A), both of which contain extrachromosomal double minutes (DMs) (Table 1), and the Y79 cell line which contains a homogeneously staining region (HSR), all possessed a 2-kilobase (kb) *EcoRI* fragment in addition to the

13.5-kb *c-myc* fragment which demonstrates homology to the *v-myc* probe (data not shown). Both DMs and HSRs have been implicated in gene amplification of the N-myc in neuroblastoma^{17,18}. Our results suggest that the retinoblastomas LA-RB70, LA-RB63-1A and Y79 show a similar amplification of the N-myc gene. Because the homology between *c-myc* and N-myc is limited^{17,18}, the N-myc sequences must be amplified to be detected by the *v-myc* probe in the Southern blotting. However, we found no gross abnormalities of the *c-myc* gene in these samples.

To confirm that the 2-kb *EcoRI* fragment is the N-myc gene, the pNb-1 plasmid (provided by Dr M. Bishop), which contains part of the known N-myc gene, was used as probe. We found that LA-RB70, LA-RB63-1A and the Y79 cell lines do indeed contain one amplified 2-kb *EcoRI* fragment which hybridizes with the pNb-1 probe (Fig. 1a). To exclude the possibility of technical artefacts, the pT24-C (c-H-ras) probe was also used in the same blot. Two *EcoRI* fragments of about 15 and 9 kb were hybridized with approximately equal intensity in every lane of DNA (data not shown). The extent of the amplification of the N-myc gene was determined by applying different amounts of DNA for Southern blotting. As Fig. 1b shows, compared with the level of N-myc gene in normal fibroblasts or other tumours, LA-RB70 and Y79 contain a 100–200-fold amplification of the N-myc gene and LA-RB63-1A contains a 10–20-fold amplification. Amplification of the N-myc gene in Y79 cells has also been described by Kohl *et al.*¹⁸. As the Y79 cell line has been in culture for many years, the amplification of the N-myc gene may be a result of clonal selection in culture rather than amplification in the original cells. However, the fact that the gene is amplified in the LA-RB70 and LA-RB63-1A tumours indicates that it can be amplified in the primary retinoblastoma or early passaged tumour cells.

Meanwhile, we prepared and analysed DNA from the fibroblasts of three patients with retinoblastomas—LA-RB63-1A (a patient whose tumour showed amplification of the N-myc gene), LA-RB69 and LA-RB73—as well as from fibroblasts of one normal human embryo. No amplification of the N-myc gene was found in these cells (Fig. 1c). These results indicate that as

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Fig. 1 Southern blot analysis of DNA from retinoblastomas and normal fibroblasts, probed with 32 P-labelled pNb-1 DNA. **a**, Amplification of N-myc gene in retinoblastoma DNA. A 2-kb *Eco*RI resistant fragment representing the N-myc gene was hybridized with pNb-1 probe in all DNA samples. Three cases, Y79, LA-RB70 and LA-RB63-1 contained an amplified N-myc gene. In addition to the 2-kb fragment, a 3-kb fragment of LA-RB73 was also hybridized. The significance of this hybridization is not known. **b**, Determination of the N-myc gene amplification fold. Different amounts of DNA were digested with *Eco*RI, and following Southern analysis, probed with pNb-1. Fibroblast DNA from the LA-RB63 patient was used as a standard for comparison. Part of the blot containing the 2-kb fragment region is shown in the figure. **c**, Normal fibroblast DNA from retinoblastoma patients had no amplification of the N-myc gene. Fibroblast DNA from three retinoblastoma patients, including one whose retinoblastoma contained N-myc amplification, one normal fetus and one adult lymphocyte DNA were subjected to Southern analysis, then probed with pNb-1 as described below. In the same blot, an equal amount of Y79 DNA was used for positive control.

Methods: Genomic DNA was prepared by the standard method²⁵ and $\sim 10 \mu\text{g}$ of each DNA sample were digested by *Eco*RI to completion, electrophoresed through 0.8% agarose and blotted onto nitrocellulose. Hybridization with nick-translated 32 P-labelled pNb-1 DNA (specific activity 2.5×10^8 c.p.m. μg^{-1}) was in 40% formamide, $5 \times \text{SSC}$ at 42°C for 36–48 h. Washing was done in $0.2 \times \text{SSC}$, 0.5% SDS at 55°C .

N-myc gene amplification occurred solely in tumour tissue, expression of this sequence could have an aetiological role in retinoblastoma formation.

It remained possible that abnormalities of other oncogenes existed in the eight tumours in which N-myc was not amplified at the DNA level. However, when *ras*, *ets*, *myb*, *erb* and *fps* were used as probes¹⁰ to determine if there had been any rearrangements or other changes of these oncogenes, no gross abnormalities were found in any tumour (data not shown), although we cannot exclude the possibility that a point mutation has occurred in those genes which could be important for tumour formation.

Gene amplification is only one of several mechanisms for increasing gene expression¹⁹. To determine whether there was increased expression of N-myc in retinoblastoma at the mRNA level and whether this expression is specifically associated with gene amplification, we prepared RNA from 10 individual tumours, the Y79 cell line, four different fibroblast samples, one human fetal retina and one bovine retina. This RNA was then used to perform RNA dot analysis²⁰ using the pNb-1 as probe. We found that the Y79 cell line and four retinoblastomas (LA-RB63-1, LA-RB70, LA-RB78 and LA-RB109) expressed about 100-fold higher levels of N-myc mRNA than did retinal cells. The other tumours also had elevated levels of N-myc mRNA expression. However, N-myc expression was essentially undetectable in the fibroblasts (Fig. 2).

The LA-RB63-1A and LA-RB70 tumours as well as the Y79 cell line contained an amplified N-myc gene and consequently the presence of a high level of N-myc mRNA was anticipated. However, the other tumours did not contain detectable amplification of the N-myc gene, yet expressed a high level of N-myc mRNA, varying within four to fivefold. We do not know the significance of this difference. This increased expression might be due to the loss or inactivation of both alleles of a regulatory gene or a mutation at the regulatory site of the N-myc gene. If the first possibility is true, the *Rb* gene in 13q14 might actually be a regulator of N-myc expression.

We also analysed mRNA from neuroblastoma cell lines LA-N-1, LA-N-5 and SKN-SH as controls. The LA-N-1 and LA-N-5 cells showed a high level of N-myc expression¹⁸ whereas two SKN-SH cell line variants contained neither amplified levels of the N-myc gene nor increased mRNA expression (Fig. 2). We emphasize that N-myc amplification appears to occur primarily

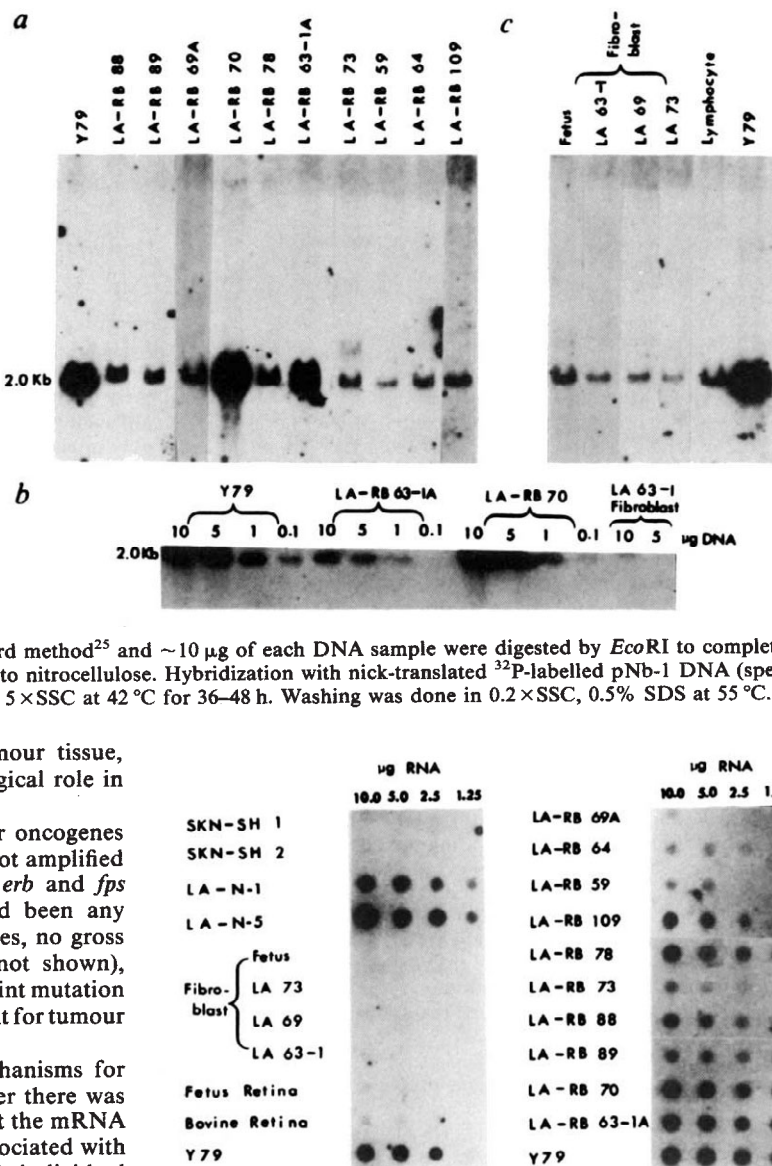


Fig. 2 Dot analysis of RNA from retinoblastoma, fibroblast, neuroblastoma and retina cells. Total cytoplasmic RNA was prepared by a method using Nonidet-P40 to lyse the cell membrane, followed by phenol-chloroform extraction²⁵. RNA was precipitated by ethanol and quantitated with A_{260} and directly dotted onto a nitrocellulose membrane in $20 \times \text{SSC}$. Hybridization was in 50% formamide and $5 \times \text{SSC}$ at 42°C with pNb-1 as probe. Washing was done in $0.2 \times \text{SSC}$ and 0.5% SDS at 60°C . Due to the limited amount of samples, some of the lanes contain RNA only in the first one or two dots.

in the advanced metastatic stages of neuroblastoma²¹, which implies that the N-myc gene may be involved in the progression of malignancy in this disease. In contrast, except for LA-RB78 which was obtained from a rib metastasis, all other retinoblastomas with increased N-myc either at the gene or mRNA level were obtained as relatively small non-metastatic intraocular tumours. As these tumours included both hereditary (bilateral) and non-hereditary (most unilateral) cases (Table 1), the possibility exists that increased N-myc expression has a more fundamental role in retinoblastoma formation.

Although the N-myc gene has a limited sequence homology to the c-myc gene, the oncogenic potential of this gene is unknown. A suitable *in vitro* assay system to test the transforming ability of the N-myc gene should provide information on this issue. Nevertheless, the positive correlation between N-myc

Table 1 Summary of retinoblastomas used in the present study

Tumour designation	Tumour material used	Bilateral (B) or unilateral (U) case	Presence of DM or HSR	N-myc gene amplification	N-myc mRNA expression
LA-RB59	SQP 1*	U	—	—	+
LA-RB63-1A	SQP 1	B	+	10–20	+++
LA-RB64	SQP 1	U	—	—	+
LA-RB69A	EP 9†	B	—	—	+
LA-RB70	Primary	U	+	100–200	+++
LA-RB73	Primary	B	—	—	+
LA-RB78	SQP 2	U	—	—	+++
LA-RB88	Primary	B	—	—	++
LA-RB89	Primary	U	—	—	++
LA-RB109	Primary	U	—	—	+++
Y79	Cell line	B	+	100–200	+++

Tumours LA-RB59 to LA-RB78 have been described in ref. 8. Tumour LA-RB78 was obtained from rib metastasis rather than intraocular tumour. DM, extra-chromosomal double minutes; HSR, homogeneously staining region; +, high level of expression; —, not found.

*Subcutaneous passage in nude mice.

†Eye passage in nude mice.

amplification in neuroblastoma and N-myc expression in the retinoblastoma strongly suggests that the N-myc gene may imply that the N-myc gene may be involved in the progression of malignancy in this disease. In contrast, except for LA-RB78 which was obtained from a rib metastasis, all other retinoblastomas with increased N-myc either at the gene or mRNA level were obtained as relatively small non-metastatic intraocular tumours. As these tumours included both hereditary (bilateral) and non-hereditary (most unilateral) cases (Table 1), the possibility exists that increased N-myc expression has a more fundamental role in retinoblastoma formation.

Several human tumours, including retinoblastoma, Wilms' tumour and neuroblastoma, are associated with specific chromosomal deletions²³. The recessive nature of a relevant gene for tumour formation in the deleted band has been demonstrated for the *Rb* gene^{1,3,4} and is likely for Wilms' tumour²⁴. We speculate that recessive genes like the *Rb* gene in these tumours may have a regulatory role to modulate a set of proto-oncogenes. This hypothesis can be tested when the molecular nature of the *Rb* gene is elucidated.

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- Murphree, A. L. & Benedict, W. F. *Science* **223**, 1028–1033 (1984).
- Sparkes, R. S. *et al. Science* **219**, 971–973 (1983).
- Benedict, W. F. *et al. Science* **219**, 973–975 (1983).
- Cavenee, W. *et al. Nature* **305**, 779–784 (1983).
- Knudson, A. G. *Proc. natn. Acad. Sci. U.S.A.* **68**, 820–823 (1971).
- Comings, D. E. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3324–3328 (1973).
- Godbout, R., Dryja, T. P., Squire, J., Gallie, B. I. & Phillips, R. A. *Nature* **304**, 451–453 (1983).
- Benedict, W. F., Banerjee, A., Mark, C. & Murphree, A. L. *Cancer Genet. Cytogenet.* **8**, 311–333 (1983).
- Cooper, G. M. *Science* **218**, 801–806 (1983).
- Bishop, J. M. A. *Rev. Biochem.* **52**, 301–354 (1983).
- Santos, E., Tronick, S. R., Aaronson, S. A., Pulciani, S. & Barbacid, M. *Nature* **298**, 343 (1982).
- Land, H., Parada, L. A. & Weinberg, R. A. *Science* **222**, 771–778 (1983).
- Leder, P. *et al. Science* **222**, 765–771 (1983).
- Perucho, M. *et al. Cell* **27**, 467 (1981).
- Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
- Lee, W.-H., Liu, C.-P. & Duesberg, P. H. *J. Virol.* **44**, 401–412 (1982).
- Schwab, M. *et al. Nature* **305**, 245–248 (1983).
- Kohl, N. Z. *et al. Cell* **35**, 359–367 (1983).
- Schimke, R. T. (ed.) *Gene Amplification* (Cold Spring Harbor Laboratory, New York, 1982).
- White, B. & Bancroft, F. *J. biol. Chem.* **257**, 8569–8572 (1982).
- Brodeur, G. M., Seeger, R. C., Schwab, M., Varmus, H. E. & Bishop, J. M. *Science* (in the press).
- Kyritsis, A. P., Tsokos, M., Triche, T. J. & Chader, G. J. *Nature* **307**, 471–473 (1984).
- Yunis, J. J. *Science* **221**, 227–236 (1983).
- Koufos, A. *et al. Nature* **309**, 170–172 (1984).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).

Identification of a form of the avian erythroblastosis virus *erb-B* gene product at the cell surface

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Avian erythroblastosis virus (AEV) induces both erythroblastosis and fibrosarcoma in chickens¹. The viral oncogene responsible for these diseases, *erb*, is divided into two regions, *erb-A* and *erb-B*, although recent evidence suggests that it is primarily the *erb-B* gene product that is responsible for the transforming activity^{2–5}. The *erb-B* gene product has been reported previously to be a membrane glycoprotein of 68,000 molecular weight (MW), gp68^{*erb-B*} (refs 6, 7). However, we show here that gp68^{*erb-B*} is an intracellular precursor which is modified further to a 74,000 MW protein, gp74^{*erb-B*}. By the criteria of resistance to digestion with endoglycosidase H, subcellular fractionation and inhibition of biosynthesis by the ionophore monensin, gp74^{*erb-B*} appears to be located at the cell surface. Recently, a comparison of the *erb-B* sequence⁸ with that of the epidermal growth factor (EGF) receptor has shown that these two genes are highly homologous⁹, and that *erb-B* appears to represent a truncated form of this growth factor. In light of these data the identification of gp74^{*erb-B*} at the plasma membrane suggests that this may be the functionally important form of the *erb-B* gene product.

Identification of homology between the *erb-B* gene product and the tyrosine kinase domains of other viral oncogenes⁸ prompted us to propose that the *erb-B* gene product may function as a growth factor receptor^{6,10,11}. Our hypothesis has recently been confirmed with the demonstration that *erb-B* shows near identity with the carboxy-terminal third of the human EGF receptor⁹, and suggests that *erb-B* functions as a truncated growth factor receptor. However, this highlighted the major problem with our original hypothesis, namely the inability to detect a form of the *erb-B* glycoprotein that exhibited the hallmarks of a classic plasma membrane protein, for if *erb-B* functions as a growth factor receptor it should lie in the plasma membrane. It was therefore possible that a more mature form of the *erb-B* protein existed which we had failed to identify. We considered this highly possible, as our antisera raised in tumour-bearing rats recognized both the *erb-A* and *erb-B* proteins, and a form of the *erb-B* protein could be obscured by the *erb-A*-encoded protein p75^{*erb-A*} (ref. 12).

While screening antiserum from tumour-bearing rats which had been injected with lower numbers of AEV-transformed rat-1 cells than used previously⁶, we identified 10 out of 60 animals tested which produced *erb-B*-specific antibodies. These antisera were used to analyse the *erb-B* proteins in greater detail. Immunoprecipitation analysis (Fig. 1a, track 2) revealed that these antisera precipitated only the *erb-B* proteins gp66 and gp68 from the AEV-transformed erythroblasts, whereas our original tumour-bearing antisera precipitated both the p75^{*erb-A*} and the *erb-B* proteins (Fig. 1a, track 3). Normal rat serum did not precipitate any of these proteins (Fig. 1a, track 4).

Our initial aim in preparing *erb-B*-specific antisera was to identify forms of the *erb-B* glycoprotein that had gone undetected in our previous studies. An examination of the ³⁵S-methionine-labelled proteins precipitated by the *erb-B*-specific serum failed to reveal any additional proteins (Fig. 1a, track 2). However, when the AEV-transformed erythroblasts were labelled for 16 h with ³H-glucosamine, an additional protein of 74,000 MW, gp74, was obtained (Fig. 1a, track 1). This protein

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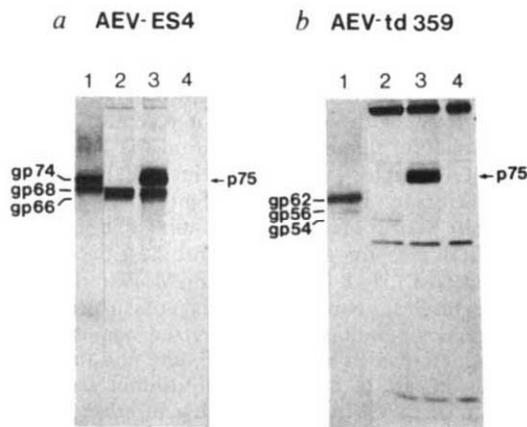


Fig. 1 Identification of *erb-B*-specific antiserum. Erythroblasts transformed by AEV-ES4 (panel *a*) or an isolate of AEV-td359 recovered from an infected chicken (panel *b*) were labelled with 100 μ Ci of 35 S-methionine for 30 min (tracks 1) or 500 μ Ci 3 H-glucosamine for 16 h (track 1). Immune precipitates were formed using rat anti-*erb-B* serum (tracks 1, 2), rat anti-*erb-A* plus *erb-B* (track 3) or normal rat serum (track 4). The precipitates were analysed by SDS-polyacrylamide gel electrophoresis and the labelled proteins were visualized by fluorography. The films were exposed for 2 days for tracks 2–4, and 3 weeks for track 1. Anti-*erb* serum was prepared by injection of 10^5 – 10^6 AT1a cells intraperitoneally into 10–14-day-old (Fischer $\times$ Wistar)F₁ rats as described previously⁶. The sera were collected 10–12 weeks later and tested for anti-*erb* activity by immunoprecipitation.

was precipitated by the *erb-B*-specific serum from extracts of AEV-transformed erythroblasts (Fig. 1*a*, track 1) or fibroblasts (data not shown), suggesting that it was either an *erb-B*-encoded protein or a cellular protein. To distinguish between these possibilities, we used AEV-rtd359, a back mutant of AEV-td359 which has recovered the ability to transform erythroblasts but which has a deletion in *erb-B* and synthesizes *erb-B* proteins that are 12,000 daltons smaller than the wild-type protein (T. Graf, M.J.H., H.B. and B. Vennström, unpublished observations). Therefore, if gp74 was in fact encoded by *erb-B*, one would expect a glucosamine-labelled protein of a smaller size. The 35 S-methionine-labelled *erb-B* protein of AEV-rtd359 had MWs of 54,000 and 56,000 (Fig. 1*b*, track 2), the 3 H-glucosamine-labelled proteins were of MWs 56,000 and 62,000 and no gp74 was identified in these cells (Fig. 1*b*, track 1). These data show that the gp74 glycoprotein is not a cellular protein, as it would have been detected in all the transformed erythroblasts. In addition, because there is a 3 H-glucosamine-labelled protein 6,000–10,000 daltons larger than the largest 35 S-methionine-labelled *erb-B* protein in both AEV strains, the data strongly suggest that these larger forms are products of the *erb-B* gene.

The presence of 35 S-methionine-labelled gp74 was investigated by pulse-chase analysis and precipitation of the lysates with *erb-B*-specific antiserum. Following a 30-min pulse-label, both gp66 and gp68 were found in the cells (Fig. 2*a*), and during the chase period gp66 was processed into gp68, as shown previously⁶. However, after a 1-h chase period, gp74 was also found in the cells, the amount increasing after 2 h and reaching a steady-state level by 4 h (Fig. 2*a*). Interestingly, the amount of label found in gp74 accounts for only ~25% of that initially detected in gp66 and gp68 (Fig. 2*a*). This means either that only 25% of the newly synthesized *erb-B* protein was processed into the gp74 form and the rest was rapidly degraded intracellularly or that the gp74 protein is being sequestered to a pool that is no longer detectable.

Digestion with the enzyme endoglycosidase H (endo-H)¹³ had shown previously that both gp66 and gp68 were sensitive to digestion by this enzyme⁶. To see if gp74 was also sensitive to digestion, cells were labelled for 30 min with 35 S-methionine and chased for 3 h to allow the formation of gp74. Figure 2*b*

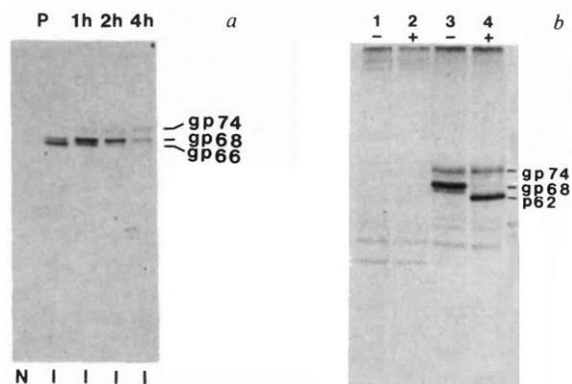


Fig. 2 Pulse-chase and endo-H digestion analysis of the *erb-B* glycoprotein. *a*, Erythroblasts transformed by AEV-ES4 were labelled for 30 min with 100 μ Ci 35 S-methionine. The cells were then pelleted and resuspended in complete growth medium. An aliquot of cells was removed for time point zero (P) and subsequent aliquots were removed after 1, 2 or 4 h. Detergent lysates were prepared and immune precipitates formed with either normal rat serum (N) or rat anti-*erb-B* serum (I). *b*, AEV-ES4-transformed erythroblasts were labelled for 30 min with 35 S-methionine, pelleted and resuspended in complete growth medium. The cells were then incubated for a further 3 h, collected and detergent lysates prepared. Immune precipitates were then formed with either normal rat serum (tracks 1, 2) or rat anti-*erb-B* serum (tracks 3, 4). The immune precipitates were then halved and one-half was treated with endo-H (+) as described previously¹⁵. The other half of the sample was treated in exactly the same way but without the addition of endo-H (-). All samples were analysed on a 10% SDS-polyacrylamide gel.

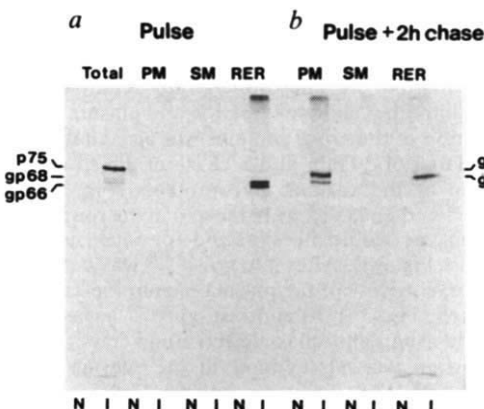


Fig. 3 Localization of gp74^{*erb-B*} to the plasma membrane. AEV-ES4-transformed erythroblasts were labelled for 30 min with 500 μ Ci of 35 S-methionine, and pelleted at 1,500g for 5 min. They were then resuspended in growth medium, and an aliquot (~10%) of the cells was taken to check the total incorporation (Total). From the remainder, half were removed for time point zero (*a*), the rest were incubated for a further 2 h (*b*). Both cell populations were then fractionated into plasma membrane (PM), smooth membrane (SM) and rough endoplasmic reticulum (RER)-enriched membrane populations, the fractions were characterized by buoyant density, RNA content and the presence of marker enzymes as described previously^{6,16,17}. The membranes were dissolved in 0.5% NP40, 0.5% DOC, 50 mM Tris-HCl pH 8.1, 100 mM NaCl, and immune precipitates formed with either normal rat serum (N) or anti-*erb-A* + *B* serum (I). The precipitates were analysed on a 10% SDS-polyacrylamide gel and the proteins visualized by fluorography after 10 days exposure.

shows that the gp68 form is still sensitive to endo-H, whereas the gp74 form is completely resistant to this enzyme, indicating that the carbohydrate chains on gp74^{*erb-B*} have been processed into the complex form.

Analysis of gp66^{*erb-B*} and gp68^{*erb-B*} by subcellular fractionation had localized these proteins to the microsomal fraction of the cell⁶. However, these previous studies had not distinguished

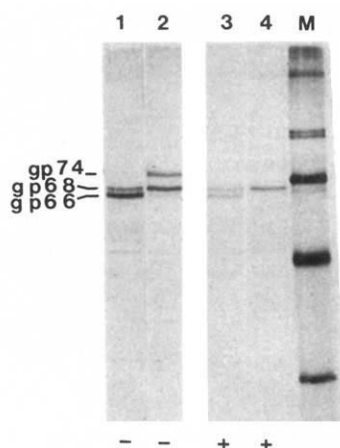


Fig. 4 Monensin inhibits the formation of gp74^{erb-B}. AEV-transformed erythroblasts were preincubated for 2 h in the absence (–) or presence (+) of 10 μ M monensin (a kind gift of Dr M. Owen). Cells were then labelled for 30 min with ³⁵S-methionine, one population in the absence and the other in the presence of 10 μ M monensin. The cells were pelleted and half taken from each population for the pulse time point (tracks 1, 3), the rest being resuspended in growth medium and incubated for a further 2 h, again in the absence (–) or presence (+) of 10 μ M monensin (tracks 2, 4). Detergent extracts were prepared and immune precipitates formed with anti-*erb-B* serum. The precipitates were analysed on a 10% SDS-polyacrylamide gel, and the proteins visualized by fluorography. Exposure time 5 days. M denotes ¹⁴C-labelled molecular weight standards from Amersham of 200,000, 94,000, 68,000, 45,000 and 30,000.

in which membrane populations the proteins resided, nor had any biosynthetic pathway for the processing of the *erb-B* glycoprotein been elucidated. We therefore extended this analysis by subfractionating the microsomes into rough endoplasmic reticulum, a smooth membrane fraction and plasma membranes. The localization of the *erb-B* proteins was also analysed following a pulse-label of 30 min and a chase of 2 h to monitor any redistribution of the various glycoproteins (Fig. 3). Initially, both gp66^{erb-B} and gp68^{erb-B} were located in the rough endoplasmic reticulum, as would be expected for intrinsic membrane glycoproteins (Fig. 3a). After 2 h, gp74^{erb-B} was detected, but it was located exclusively in the plasma membrane-enriched fraction of the cell (Fig. 3b). In contrast, gp68^{erb-B} was still mainly located in the rough endoplasmic reticulum fractions although a minor portion was also found in the plasma membrane-enriched fraction (Fig. 3b).

Monensin is a sodium ionophore which alters the intracellular transport of membrane glycoproteins¹⁴. Because gp74 seems to be the plasma membrane form of the *erb-B* gene, it was of interest to test whether monensin would inhibit its processing. A pulse-chase analysis performed in the presence and absence of 10 μ M monensin revealed that in the absence of the ionophore the *erb-B* glycoproteins were processed to the gp74 form as usual (Fig. 4, tracks 1, 2). In contrast, although gp66^{erb-B} was processed to gp68^{erb-B} in the presence of monensin, no gp74^{erb-B} was produced (Fig. 4, track 4). Therefore, monensin completely inhibits the processing of gp68^{erb-B} to gp74^{erb-B}.

The production of antisera specific for the *erb-B* product allowed us to identify and characterize a previously unrecognized form of the *erb-B* glycoprotein, namely gp74^{erb-B}. Moreover, the observation that gp68^{erb-B} is sensitive to endo-H whereas gp74^{erb-B} is not, implies that the processing of gp66^{erb-B} to gp68^{erb-B} takes place in the rough endoplasmic reticulum whereas the processing of gp68^{erb-B} to gp74^{erb-B} probably occurs in some compartment of the Golgi complex. The sensitivity of the processing of gp68^{erb-B} to gp74^{erb-B} to the presence of 10 μ M monensin, an ionophore which interferes with the passage of certain glycoproteins through the Golgi apparatus¹⁴, is consistent with this hypothesis. Taken together with the cell fractionation studies, these data imply that gp74^{erb-B} is the plasma

membrane form of the *erb-B* gene product; a similar molecule has recently been identified by M. L. Privalsky and J. M. Bishop (personal communication).

With regard to the finding that the *erb-B* gene appears to be a truncated form of the growth factor receptor, most of the coding information removed comes from the amino-terminal end of the EGF receptor⁹, which would include the EGF binding site. This helps to explain two recent observations. First, although there is near identity at the amino acid level between *erb-B* and EGF receptor, antisera against these two proteins do not cross-react (M.J.H., J. Downward and M. Waterfield, unpublished observations). However, this is not surprising as the anti-EGF receptor antibodies are directed against its amino terminus, which is missing from *erb-B*, and antibodies against the external domain of *erb-B* probably cannot gain access to this region in the EGF receptor because in *erb-B* the domain has a MW of only ~12,000 compared with 115,000 for the EGF receptor. Second, no kinase activity associated with *erb-B* has been detected in the standard immune complex assay¹⁰. This is consistent with its having lost the binding site for the growth factor, interaction with which would presumably stimulate activity. However, this does not mean that *erb-B* could not function as a kinase in certain specific conditions, and this enzymatic function may yet be involved in *erb-B*-induced cell transformation.

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1. Graf, T. & Beug, H. *Cell* **34**, 7–9 (1983).
2. Frykberg, L. *et al.* *Cell* **32**, 227–238 (1983).
3. Sealy, L., Privalsky, M. L., Moscovici, G., Moscovici, C. & Bishop, J. M. *Virology* **130**, 155–178 (1983).
4. Yamamoto, T., Hihara, H., Nishida, T., Kawai, S. & Toyoshima, K. *Cell* **34**, 225–232 (1983).
5. Fung, Y. K. T., Lewis, W. G., Kung, H. J. & Crittenden, L. B. *Cell* **33**, 357–368 (1983).
6. Hayman, M. J. *et al.* *Cell* **32**, 579–588 (1983).
7. Privalsky, M. L., Sealy, L., Bishop, J. M., McGrath, J. P. & Levinson, A. D. *Cell* **32**, 1257–1267 (1983).
8. Yamamoto, T. *et al.* *Cell* **35**, 71–78 (1983).
9. Downward, J. *et al.* *Nature* **307**, 521–527 (1984).
10. Hayman, M. J. & Beug, H. *Cold Spring Harb. Conf. Cell Proliferation and Cancer* **9** (in the press).
11. Beug, H. & Hayman, M. J. *Cell* **36**, 963–972 (1984).
12. Hayman, M. J., Royer-Pokora, B. & Graf, T. *Virology* **92**, 31–45 (1979).
13. Tarentino, A. L. & Maley, F. *J. Biol. Chem.* **249**, 811–817 (1974).
14. Tartakoff, A. *Cell* **32**, 1026–1029 (1983).
15. Owen, M. J., Kissinger, A. M. & Lodish, H. F. *J. Biol. Chem.* **255**, 9678–9684 (1980).
16. Hayman, M. J. *Virology* **85**, 475–486 (1978).
17. Wallach, D. F. H. & Kamat, V. B. *Meth. Enzym.* **8**, 164–172 (1966).

Molecular cloning of the whole biosynthetic pathway of a *Streptomyces* antibiotic and its expression in a heterologous host

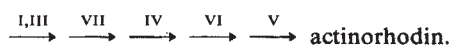
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The application of molecular cloning to antibiotic-producing microorganisms should lead to enhanced antibiotic productivity and to the biosynthesis of novel antibiotics by *in vitro* interspecific recombination^{1,2}. To allow such approaches, the genes for antibiotic synthesis must be isolated, analysed and perhaps modified. Certain *Streptomyces* species produce nearly two-thirds of the known natural antibiotics³; the recent development of cloning systems in the genus^{4–7} makes it possible to isolate and analyse *Streptomyces* genes. However, antibiotics are metabolites which require sets of several enzymes for their synthesis and attempts to isolate the corresponding genes have so far yielded clones carrying either individual genes of the set, or only incomplete gene sets^{8–11}. We describe here the isolation of a large continuous segment of *Streptomyces coelicolor* DNA which apparently carries

the complete genetic information required for synthesis of an antibiotic, actinorhodin, from simple primary metabolites. Not only can the cloned DNA 'complement' all available classes of actinorhodin non-producing mutants of *S. coelicolor* but, on introduction into a different host, *Streptomyces parvulus*, it directs the synthesis of the antibiotic. The tendency for the genes for antibiotic synthesis to be clustered together on the chromosomes of *Streptomyces* species¹² and the availability of plasmid vectors which can carry stable inserts of DNA larger than 30 kilobase pairs (kb) and which can be introduced efficiently into *Streptomyces* protoplasts, suggest that the experiments described have general significance for this area of biotechnology.

Actinorhodin¹³ belongs to a class of antibiotics named isochromanonequinones which also includes granaticin¹⁴, kalafungin¹⁵ and the nanaomycins¹⁶. It is derived biosynthetically from acetate units (acetyl coenzyme A) through the polyketide pathway¹⁷. Rudd and Hopwood¹⁸ isolated 76 actinorhodin non-producing mutants (*act*) and classified them into seven phenotypic classes, where each class represented lesions in a different biosynthetic step. Mutants in six of the classes co-synthesized actinorhodin in combination with other mutants, allowing a tentative biosynthetic sequence to be deduced, as follows:



Mutants of class II failed to co-synthesize actinorhodin in any combination and perhaps included polar and regulatory mutations. At least one mutation of each class was mapped to the same segment of the *S. coelicolor* chromosome¹⁸. The actinorhodin system therefore offered the possibility of cloning a DNA fragment encoding a complete biosynthetic pathway provided that a suitable vector was available, with the eventual aim of analysing its physical organization and regulation.

Reconstruction experiments indicated that *Act*⁺ colonies could be detected easily among a large number of *act* colonies by the formation of the blue diffusible actinorhodin when grown on R2YE medium⁶. 'Complementation' of an *act* mutant, detected by restoration of the pigmented phenotype, was therefore used as the screen for desired clones. A mutant of *act* class V, blocked in a late step of the biosynthesis (and also carrying a *red* mutation to abolish production of the other pigment, undecylprodigiosin¹⁹) was used as the recipient for cloned DNA from the wild-type *act*⁺ strain.

The plasmid SCP2*, a 31-kb sex factor of *S. coelicolor*^{20,21},

has recently been developed into a series of cloning vectors by deletion of dispensable DNA and by addition of drug resistance markers²². One such vector is pIJ922 (25 kb) which contains a gene for thiostrepton resistance (*tsr*) from *Streptomyces azureus*⁶ and has single sites for several restriction enzymes, including *Bam*HI, within a non-essential region. DNA of pIJ922, purified by CsCl-ethidium bromide density gradient centrifugation²¹, after alkaline lysis²³, was digested with *Bam*HI. After phenol extraction, 0.5 µg of the linearized plasmid was mixed with 5 µg of chromosomal DNA from an *act*⁺ *S. coelicolor* strain which had been partially digested with *Mbo*I and size-fractionated in a sucrose density gradient²⁴ to give fragments in the size range 15–30 kb. The DNA mixture, after ethanol precipitation, was dissolved in T4-DNA ligase buffer and ligated²⁵ at a final DNA concentration of 50 µg ml⁻¹. The ligated DNA was re-precipitated and dissolved in 25 µl TE (10 mM Tris-HCl, 1 mM EDTA, pH 8). An aliquot containing 4.4 µg of ligated DNA was used to transform²⁵ protoplasts of *S. coelicolor* SCP2⁻ strain JF4 (*proA1 argA1 cysD18 strA1 act109* (V) *redE60*)⁸. After allowing 20 h for phenotypic expression of thiostrepton resistance, the R2YE regeneration medium plates were overlaid with soft nutrient agar (Difco) containing thiostrepton to give a final concentration in the entire plates of 50 µg ml⁻¹.

Two blue colonies (presumptive clones) were detected among a total of ~8,000 transformants growing on 20 plates. Isolation of CCC DNA from the colonies revealed recombinant plasmids (named pIJ2300 and pIJ2301); each was introduced by transformation into *act* mutants of each of the seven classes, with the results shown in Table 1. pIJ2300 complemented all classes except VI, while pIJ2301 complemented classes V, VI and VII. Restriction maps of the plasmids revealed inserts of 34 and 16 kb in pIJ2300 and pIJ2301 respectively, with an apparently overlapping region of 12 kb (Fig. 1).

The entire insert of pIJ2300 was bounded by *Bam*HI sites. Moreover, preliminary subcloning experiments showed that class VI mutations could be complemented by a 4.5-kb *Bam*HI fragment uniquely present at the left-hand end of pIJ2301 (Fig. 1) and presumably the most distal region carrying biosynthetic genes. This suggested a strategy for constructing a single clone able to complement all available *act* mutants, and perhaps containing the entire chromosomal region responsible for actinorhodin biosynthesis. A mixture of pIJ2300 partially digested with *Bam*HI and pIJ2301 completely digested with *Bam*HI (plus *Eco*RI to prevent re-formation of pIJ2301) was ligated and used to transform protoplasts of strain JF3 (*act* class

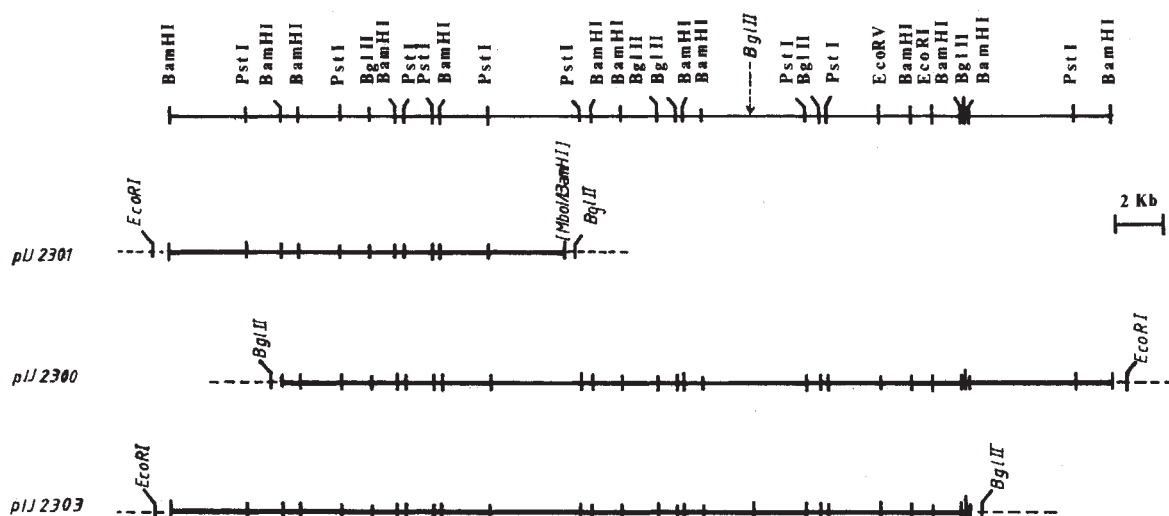


Fig. 1 Comparative restriction maps of *act*⁺ clones pIJ2301, pIJ2300 and pIJ2303. The maps of pIJ2300 and pIJ2303 were derived from the maps of overlapping fragments subcloned into SCP2*-derived vectors. In addition to loss of a 6-kb *Bam*HI fragment from the right-hand end of pIJ2300, pIJ2303 carries a new *Bgl*II site (arrow). From the results of subcloning experiments, this discrepancy does not affect the ability of pIJ2303 to 'complement' all of the *act* mutants tested, and may be explicable by homologous recombination between pIJ2300 (with wild-type DNA) and the chromosome of the *act* mutant in *S. coelicolor* JF4, giving rise to a heterogeneous plasmid population. Dashed lines indicate pIJ922 vector DNA, with the *Eco*RI and *Bgl*II sites shown for orientation.

Table 1 Complementation test of *act*⁺ recombinant plasmids with *act* *S. coelicolor* mutants and expression of the clones in *S. parvulus*

Recipient strain		Blue pigment produced by strains carrying		
John Innes stock no.	act class	pIJ2300	pIJ2301	pIJ2303
<i>S. coelicolor</i> *				
JF1	II	+	—	+
TK17	I	+	—	+
TK18	III	+	—	+
B257	VII	+	+	+
TK16	IV	+	—	+
JF3	VI	—	+	+
JF4	V	+	+	+
<i>S. parvulus</i> 2283†	—	—	—	+
<i>S. parvulus</i> 2618‡	—	—	—	+

* All the strains are mutational and recombinational derivatives of *S. coelicolor* A3(2); their complete genotypes are as previously reported⁸.

† Chromosomal marker: *str-1*.

‡ Chromosomal markers: *cys-1 his-5*.

VI). Of 20 blue transformants, one carried a plasmid (pIJ2303) that complemented strongly *act* mutants of all seven classes. The restriction map of pIJ2303 (Fig. 1) demonstrated insertion of the required 4.5-kb *Bam*HI fragment from pIJ2301 at the left end of the pIJ2300 insert and in the original orientation of pIJ2301. At the opposite end, pIJ2303 showed deletion of a 6-kb *Bam*HI fragment present in pIJ2300, but evidently this was not required for complementation of any of the available *act* mutants.

To determine whether pIJ2303 carried the entire set of *act* biosynthesis genes, pIJ2300, pIJ2301 and pIJ2303 were introduced by transformation into genetically marked strains of *S. parvulus* ATCC 12434. This strain does not produce actinorhodin and is not a close relative of *S. coelicolor* A3(2) as judged either by conventional taxonomy or by DNA-DNA hybridization²⁶. It is, however, a host for SCP2 plasmids. Only those transformants that grew on R2YE plates containing thiostrepton and which carried pIJ2303 were blue (Table 1). Thin-layer chromatography of methanol extracts¹⁹ of the *S. parvulus* host cultures and the same strains containing each of the three recombinant plasmids showed the presence of a blue pigment only in clones carrying pIJ2303. This blue pigment co-migrated with a corresponding pigment in extracts from *act*⁺ but not *act* *S. coelicolor* cultures and had acid-base indicator properties characteristic of actinorhodin. The amounts of the blue pigment in extracts of *S. parvulus* carrying pIJ2303 were similar to those of wild-type *S. coelicolor*. This result suggests that the entire DNA sequence corresponding to the structural genes for actinorhodin biosynthesis is carried by pIJ2303.

The success of the cloning experiments reported here may well have depended on the use of a low-copy number vector (the estimated copy number of the parent SCP2 is one per chromosome of *S. coelicolor*²¹) so that the recipient strain is not likely to be placed at a disadvantage by possible high-level expression of physiologically active gene products. This view is supported, though not proved, by our failure to isolate any *Act*⁺ clones in extensive trials with the high-copy number vector pIJ702 (ref. 27) despite the fact that this vector has proved convenient for the cloning of comparatively short segments of DNA carrying other genes^{8,28}. Vectors such as pIJ922, and other SCP2* derivatives recently developed by Lydiate²², appear to be particularly suitable for the stable maintenance of large fragments of cloned DNA. Moreover, the large recombinant plasmids are readily isolated from *Streptomyces* cultures²³, and transform *Streptomyces* protoplasts efficiently. Thus pIJ2300, of 59 kb, yielded 10⁶ transformants per µg of DNA in optimal conditions, compared with 10⁷ for the pIJ922 vector.

As the entire carbon skeleton of actinorhodin is derived from acetate¹⁷, no specialized primary metabolites are required for its biosynthesis, a factor which may have contributed to the

efficient expression of pIJ2303 in *S. parvulus*, leading to production of actinorhodin. Many important antibiotics are also polyketides—including the macrolides, tetracyclines and rifamycins. It is generally accepted that the primary carbon skeletons of polyketides are assembled on multienzyme complexes (or multifunctional enzymes) analogous to fatty acid synthetases²⁹, and the genes or gene clusters that encode polyketide synthetases may have had a common evolutionary origin, perhaps shared with fatty acid synthetases. Thus, the availability of clones carrying the presumptive actinorhodin 'synthetase' may be useful, not only as a model system, but possibly as DNA probes for the isolation of other antibiotic synthetases.

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- Hopwood, D. A. & Chater, K. F. *Phil. Trans. R. Soc. B290*, 313–328 (1980).
- Hopwood, D. A. in *β-Lactam Antibiotics* (eds Salton, M. R. J. & Shockman, G. D.) 585–598 (Academic, New York, 1979).
- Bérty, J. *Process. Biochem. Oct./Nov.*, 28–35 (1980).
- Bibb, M. J., Schottel, J. L. & Cohen, S. N. *Nature* **284**, 526–531 (1980).
- Suarez, J. E. & Chater, K. F. *Nature* **286**, 527–529 (1980).
- Thompson, C. J., Ward, J. M. & Hopwood, D. A. *Nature* **286**, 525–527 (1980).
- Bibb, M. J., Chater, K. F. & Hopwood, D. A. in *Experimental Manipulation of Gene Expression* (ed. Inoué, M.) 53–82 (Academic, New York, 1983).
- Feitelson, J. S. & Hopwood, D. A. *Molec. gen. Genet.* **190**, 394–398 (1983).
- Gil, J. A. & Hopwood, D. A. *Gene* **25**, 119–132 (1983).
- Chater, K. F. & Bruton, C. J. *Gene* **26**, 67–78 (1983).
- Hopwood, D. A., Bibb, M. J., Bruton, C. J., Feitelson, J. S. & Gil, J. A. *Trends Biotechnol.* **1**, 42–48 (1983).
- Hopwood, D. A. in *Biochemistry and Genetic Regulation of Commercially Important Antibiotics* (ed. Vining, L. C.) 1–23 (Addison-Wesley, Reading, Massachusetts, 1983).
- Brockman, H., Zeek, A., van der Merve, K. & Müller, W. *Justus Liebig's Annl. Chem.* **698**, 3575–3579 (1966).
- Carbaz, R. et al. *Helv. chim. Acta* **40**, 1262–1269 (1957).
- Hoeksema, H. & Krueger, W. C. *J. Antibiot., Tokyo* **29**, 704–709 (1976).
- Tanaka, H., Koyama, V., Nagai, T., Marumo, H. & Omura, S. *J. Antibiot., Tokyo* **28**, 868–875 (1975).
- Gorst-Allman, C. P., Rudd, B. A. M., Chang, C.-J. & Floss, H. G. *J. org. Chem.* **46**, 455–456 (1981).
- Rudd, B. A. M. & Hopwood, D. A. *J. gen. Microbiol.* **114**, 35–43 (1979).
- Rudd, B. A. M. & Hopwood, D. A. *J. gen. Microbiol.* **119**, 333–340 (1980).
- Schrempf, H., Bujard, H., Hopwood, D. A. & Goebel, W. *J. Bact.* **121**, 416–421 (1975).
- Bibb, M. J., Freeman, R. F. & Hopwood, D. A. *Molec. gen. Genet.* **154**, 155–166 (1977).
- Lydiate, D. J. thesis, Univ. East Anglia, Norwich (1984).
- Kieser, T. *Plasmid* (in the press).
- Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J. & O'Connell, C. *Cell* **15**, 687–701 (1978).
- Thompson, C. J., Ward, J. M. & Hopwood, D. A. *J. Bact.* **151**, 668–677 (1982).
- Westpheling, J. thesis, Univ. East Anglia, Norwich (1980).
- Katz, E., Thompson, C. J. & Hopwood, D. A. *J. gen. Microbiol.* **129**, 2703–2714 (1983).
- Seno, E. T., Bruton, C. J. & Chater, K. F. *Molec. gen. Genet.* **193**, 119–128 (1984).
- Packter, N. M. in *The Biochemistry of Plants* Vol. 4 (ed. Strumpf, P. K.) 535–570 (Academic, London, 1983).

Ha-ras oncogenes are activated by somatic alterations in human urinary tract tumours

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DNA-mediated gene transfer (transfection) studies using NIH 3T3 cells as recipients have demonstrated the presence of transforming genes (oncogenes) in diverse human tumours (for reviews see refs 1, 2). A large proportion of oncogenes so far detected by DNA transfection are related to the Ha-ras onc gene of Harvey (and BALB) murine sarcoma viruses (MSV)^{3–6}, Ki-ras, the oncogene of Kirsten MSV^{3,7,8}, and a third member of the ras gene family, N-ras (refs 8–12). Individual tumours of many different organs

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have been associated with the activation of members of the *ras* gene family. We now present the first systematic survey of human urinary tract tumours processed immediately after surgery, as well as normal tissues from the same patients, to detect the presence of such genes. We demonstrate activation of *Ha-ras* as an oncogene in around 10% of randomly selected urinary tract tumours as well as direct evidence that oncogene activation is the result of a somatic event which is selected for within the tumour cell population.

Urinary tract carcinoma, at any site from renal pelvis to urethra, is considered to be a gross manifestation of a generalized neoplastic change of its epithelium, the urothelium. Multiple bladder carcinomas are not uncommon at cystoscopy and detailed histological examination of surgical specimens from patients with carcinomas of the bladder, renal pelvis and ureter has revealed a high incidence of neoplastic and preneoplastic lesions at sites other than the actual tumour^{13,14}. To survey for oncogenes detectable by transfection analysis, high molecular weight DNA in sufficient quantity for analysis was obtained from 23 out of 32 randomly selected consecutive specimens of urinary tract tumours. The tumours were transitional cell carcinomas except for one adenocarcinoma.

Transforming activity was observed with two tumours. These two patients (JPT26 and JBT44) had no special features in terms of occupation, past history, or clinical or pathological characteristics of the tumour¹⁵. Moreover, there was no apparent correlation between the presence of an active oncogene and the degree of invasiveness of the tumours analysed. In each case, transforming activity survived a second cycle of transfection using high molecular weight DNAs of individual first-cycle NIH 3T3 transfectants (Table 1). Southern blotting analysis showed the presence of human repetitive sequences in all first- and second-cycle transfectants, implying that the foci observed were induced by human DNA (data not shown).

DNAs from JPT26- and JBT44-induced foci were hybridized with nick-translated probes specific to each of the *ras* oncogenes. Neither human *Ki-ras* nor *N-ras* probes detected DNA fragments other than the endogenous bands (data not shown). However, when *Bam*HI-digested transfectant DNAs were analysed for *Ha-ras*-related sequences, all tested JPT26 and JBT44 transfectants exhibited an additional 7-kilobase pair (kbp) DNA fragment (Fig. 1a). These findings identified the oncogene in each tumour as an activated allele of the *Ha-ras* proto-oncogene.

Accumulating evidence indicates that the major sites for the genetic lesions responsible for activation of *ras* oncogenes in

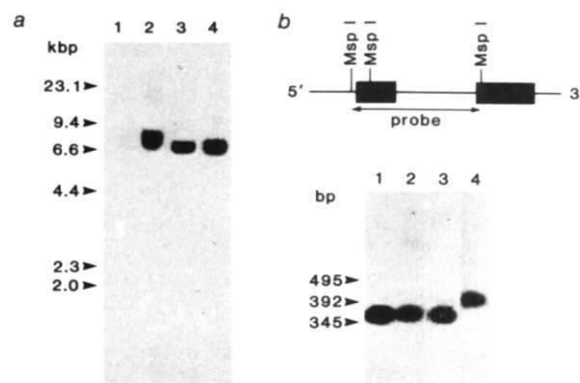


Fig. 1 Detection and identification of the human urothelial carcinoma transforming genes and their *Msp*I sensitivity at codon 12. 20 µg of high molecular weight DNA were digested with *Bam*HI (column a) or with *Msp*I and *Hpa*II (column b), electrophoresed in 1.2% or 2.0% agarose gel, respectively, transferred to nitrocellulose paper and hybridized as described previously^{6,25}. a, DNAs from NIH 3T3 cells (lane 1), human placenta (lane 2), as well as representative first-cycle transfectants of JPT26 (lane 3) and JBT44 (lane 4) tumours were hybridized with the 6.6-kbp *Bam*HI fragment of the T24 oncogene, p24-c3 (ref. 17). In these conditions, the mouse endogenous 3.5-kbp *Ha-ras*-related fragment was not observed. Co-electrophoresed DNA fragments of *Hind*III-digested λc1857 DNA served as molecular weight standards. b, The upper panel shows the *Msp*I restriction map of the human *Ha-ras* proto-oncogene. The two solid boxes indicate the first and second exons from the 5' to 3' direction. In the lower panel, DNAs from representative first-cycle transfectants of JPT26 (lane 1), JBT44 (lane 2) and T24 (lane 4) tumours, as well as human placenta DNA (lane 3) were hybridized with the T24 *Msp*I fragment shown by the arrow in the upper panel. Co-electrophoresed DNA fragments of *Hinc*II-digested ΦX174RF DNA served as molecular weight standards. The 56-bp fragment was not apparent in these experimental conditions.

Table 1 Transforming activity of DNA from human urinary tract tumours

Donor DNA	Total no. foci/total DNA tested (µg)	
	Primary	Secondary
T24 bladder carcinoma	85/400	—
JPT26 renal pelvic carcinoma*	8/320	37/240
JBT44 bladder carcinoma†	15/320	28/240

Human tumours were minced with scalpels within 4 h of surgical removal, incubated for 3 h at 37 °C with 200 µg ml⁻¹ of proteinase K in the presence of 0.5% SDS and 10 mM EDTA, extracted with phenol and precipitated with ethanol. High molecular weight DNA was further purified by treatment with RNase, proteinase K and extracted with phenol/chloroform. These DNAs, as well as DNAs from representative first-cycle NIH 3T3 transfectants, were used to transfect onto NIH 3T3 cells by the calcium phosphate precipitation technique, as described previously⁶. Foci of transformed cells were scored at 21 days. Twenty-three tumours, including four non-invasive tumours, were tested. Two of these were cytological grade 1, 11 were grade 2 and 10 were grade 3 (ref. 15). In 10 cases, bladder tumours were multiple. Eight patients had a previous history of bladder tumours.

* JPT26, a 33-yr-old male who had the right kidney and ureter removed for a papillary non-invasive transitional cell carcinoma, grade 2 of the renal pelvis.

† JBT44, a 44-yr-old male who underwent transurethral resection of recurrent multiple transitional cell carcinomas of the bladder which were grade 2 and invaded the submucosa (P1).

human tumours are localized to positions 12 and 61 in their coding sequences^{6,16-21}. An alteration at position 12 in human *Ha-ras* leads to loss of a restriction site for *Hpa*II-*Msp*I digestion, thus providing a means of molecular diagnosis of lesions at this position²². As Fig. 1b shows, DNA from JPT26 and JBT44 transfectants demonstrated a 355-base pair (bp) band (lanes 1 and 2, respectively) like that observed with human placenta DNA (lane 3), while the T24 transfectant DNA yielded the expected 411-bp band (lane 4). These results indicated that neither the JPT26 nor JBT44 oncogenes had undergone mutations affecting the 12th amino acid of its p21 coding sequence.

The recognition sequence for *Bst*NI is CCAGG, whereas the normal sequence encompassing position 61 in the *Ha-ras* proto-oncogene is CCAGG (Fig. 2)^{23,24}. Thus, any alteration in the sequence other than A to T would be expected to eliminate this *Bst*NI recognition site. As Fig. 2 shows, the electrophoretic mobilities of two *Bst*NI-digested DNA fragments hybridizing to the *Ha-ras* *Kpn*I-*Nco*I probe in a representative JBT44 transfectant (lane 4) were not distinguishable from those of a T24 transfectant, possessing a position 12 lesion (lane 1)¹⁶⁻¹⁸. The 158-bp band corresponded to the larger fragment observed with an intact *Bst*NI site at position 60-62. A transfectant containing the Hs242 oncogene, whose corresponding sequence at position 61, CCTGG (ref. 6), would also be recognized by *Bst*NI, demonstrated an identical pattern of DNA fragments (Fig. 2, lane 2). In contrast, a JPT26 transfectant DNA exhibited a different hybridizing DNA fragment, whose 173-bp size corresponded to that expected in the absence of a *Bst*NI cleavage site at position 60-62 (lane 3). Thus, these results established that the oncogene from patient JPT26 had suffered a genetic lesion affecting codons 60, 61 or 62.

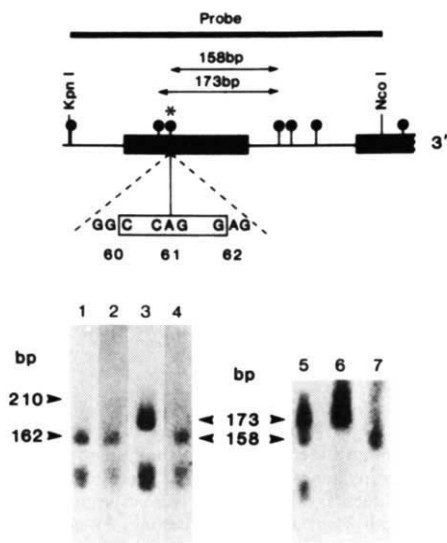


Fig. 2 *Bst*NI sensitivity encompassing codon 61 of the human *Ha-ras* gene in NIH 3T3 transfectants, JPT26 tumour and JPT26 normal DNAs. The upper panel shows the *Bst*NI restriction map of the human *Ha-ras* proto-oncogene. The two solid boxes represent the second and third exons from the 5' to 3' direction. ●, *Bst*NI cleavage sites; *, the *Bst*NI site encompassing codon 61. The nucleotide sequence surrounding this site is also shown. The numbers below the sequence indicate the amino acid codon number. The lower panel shows Southern blotting analysis of DNAs from representative NIH 3T3 transfectants of T24 (lane 1), Hs242 (lane 2), JPT26 (lanes 3, 6) and JBT44 (lane 4) tumours as well as JPT26 primary tumour (lane 5) and JPT26 normal peripheral leukocytes (lane 7). DNAs were digested with the restriction endonuclease *Bst*NI, electrophoresed in a 4% agarose–50% formamide gel²⁶, blotted to nitrocellulose paper and hybridized with the *Kpn*I–*Nco*I fragment of the human *Ha-ras* proto-oncogene as shown in the upper panel. Twice as much DNA (100 µg) was used in lane 5 as in the other lanes. The variable detection of the 128/123-bp DNA fragment (compare lanes 3 and 6) with the same JPT26 transfectant DNA reflects variability in the efficiency of transfer of low molecular weight DNA species to the nitrocellulose paper. Co-electrophoresed DNA fragments of *Hinc*II-digested ΦX174RF DNA served as molecular weight standards.

The availability of normal peripheral blood leukocytes from patients JPT26 and JBT44 made it possible to assess whether normal tissues from these patients contained normal or activated *Ha-ras* alleles. DNAs from these normal cells were assayed for transforming activity in the NIH 3T3 transfection assay. No focus-forming activity was detectable with either DNA, in conditions in which both tumour cell DNAs readily registered as positive (data not shown). When DNAs from the JPT26 primary tumour and normal peripheral blood leukocytes were analysed by a molecular genetic approach (Fig. 2), the *Ha-ras* *Kpn*I–*Nco*I probe hybridized to *Bst*NI-digested DNA fragments of 173 and 158 bp as well as to the 128/123-bp species in DNA of the primary JPT26 tumour (Fig. 2, lane 5). In contrast, normal peripheral blood leukocytes from patient JPT26 (lane 7) demonstrated only the 158-bp hybridizing DNA fragment. The 173-bp fragment specific for the altered *Ha-ras* allele present in the JPT26 transfectant (lanes 3, 6) was not detected in the normal cells. These results established that the altered *Ha-ras* allele was present in the JPT26 tumour but not in normal tissues from the same patient.

Transforming genes were detected by transfection analysis in 2 of 23 randomly selected primary human tumours of the

urothelium. In both cases, *Ha-ras* oncogenes were identified as the source of transforming activity. Neither oncogene was activated by a lesion at position 12 in the coding sequence, consistent with other studies indicating that position 12 lesions in *Ha-ras* are not commonly detected in primary human tumours²². We demonstrated a genetic alteration(s) within codon(s) 60, 61 or 62 of the *Ha-ras* coding sequence of the JPT26 tumour by a *Bst*NI restriction site polymorphism. Since position 61 is a major 'hot spot' for activation of *ras* oncogene in human tumours^{6,21}, it is likely that the genetic alteration detected at this position of the JPT26 oncogene coding sequence is responsible for its activation as a transforming gene.

It is of critical importance to establish the direct relevance of an oncogene detected in a human tumour to the neoplastic process affecting that tumour cell. In the present studies, normal cell DNA from neither of the patients whose tumours contained *Ha-ras* oncogenes possessed a transforming gene detectable by transfection analysis. By restriction enzyme analysis, it was possible to obtain direct biochemical evidence that the genetic lesion in the JPT26 oncogene was not present in normal cellular DNA of this same patient. These findings establish that this genetic alteration is the result of a somatic event which must be powerfully selected for within the tumour and, thus, likely to contribute to its development.

Our results also suggest that that *Ha-ras* oncogenes are more commonly activated in urinary tract tumours than are N- or Ki-*ras* genes. This is in contrast to haematopoietic tumours, where N-*ras* appears to be the most frequently activated *ras* oncogene^{8,12}, or lung and colon carcinoma where the Ki-*ras* oncogene appears to predominate^{3,7,19,20}. The frequency at which oncogenes were detected is likely to be an underestimate due to the inherent insensitivity of the transfection assay. Single base changes at either position 12 or 61 in the *Ha-ras* coding sequence will lead to loss of *Msp*I or *Bst*NI sites for 6/6 and 7/8 possible amino acid substitutions, respectively. Thus, a systematic survey of urinary tract carcinomas for these point mutations as well as for amplification or other rearrangements using biochemical approaches may provide a more accurate estimate of the frequency at which *Ha-ras* is activated as an oncogene in these naturally occurring human malignancies.

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- Cooper, G. M. *Science* **218**, 801–806 (1982).
- Weinberg, R. A. *Adv. Cancer Res.* **36**, 149–163 (1982).
- Der, C. J., Krontiris, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3637–3640 (1982).
- Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. *Nature* **297**, 474–478 (1982).
- Santos, E. *et al. Nature* **298**, 343–347 (1982).
- Yuasa, Y. *et al. Nature* **303**, 775–779 (1983).
- Pulciani, S. *et al. Nature* **300**, 539–542 (1982).
- Eva, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4926–4930 (1983).
- Shimizu, K., Goldfarb, M., Perucho, M. & Wigler, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 383–387 (1983).
- Shimizu, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 2112–2116 (1983).
- Hall, A., Marshall, C. J. & Spurr, N. K. & Weiss, R. A. *Nature* **303**, 396–400 (1983).
- Murray, M. J. *et al. Cell* **33**, 749–757 (1983).
- Melnicow, M. M. *J. Urol.* **68**, 261–279 (1982).
- Kakizoe, T. *et al. J. Urol.* **124**, 17–19 (1980).
- Mostofi, F. K., Sobin, L. H. & Torloni, H. *Histological Typing of Urinary Bladder Tumours* (World Health Organization, Geneva, 1973).
- Tabin, C. J. *et al. Nature* **300**, 143–149 (1982).
- Reddy, E. P., Reynolds, R. K., Santos, E. & Barbacid, M. *Nature* **300**, 149–152 (1982).
- Taparowsky, E. *et al. Nature* **300**, 762–765 (1982).
- Shimizu, K. *et al. Nature* **304**, 497–500 (1983).
- Capon, D. J. *et al. Nature* **304**, 507–513 (1983).
- Taparowsky, E., Shimizu, K., Goldfarb, M. & Wigler, M. *Cell* **34**, 581–586 (1983).
- Feinberg, A. P. *et al. Science* **220**, 1175–1177 (1983).
- Capon D. J. *et al. Nature* **302**, 33–37 (1983).
- Reddy, E. P. *Science* **220**, 1061–1063 (1983).
- Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
- Sun, Y. L., Xu, Y. Z. & Chambon, P. *Nucleic Acids Res.* **10**, 5753–5763 (1982).

Evolution of phosphofructokinase—gene duplication and creation of new effector sites

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Phosphofructokinases (PFK; EC 2.7.1.11) are tetrameric enzymes that have a key role in the regulation of glycolysis¹; as such, they are subject to allosteric activation and inhibition by various metabolites². Eukaryotic PFKs are about twice the size of prokaryotic enzymes and are regulated by a wider repertoire of effectors: for example, the subunit molecular weights of rabbit muscle (RM) PFK and *Bacillus stearothermophilus* (Bs) PFK are 82,000 and 36,000, respectively. Both enzymes are activated by ADP (or AMP), but RM-PFK is also activated by fructose bisphosphates (FBP) and inhibited by ATP and citrate. This, together with other evidence, has led to speculation that mammalian PFKs have evolved by duplication of a prokaryotic gene³⁻⁵, although previous peptide analysis⁶ failed to reveal internal homology in RM-PFK. Here we demonstrate clear homology among the N- and C-halves of RM-PFK and Bs-PFK, thus establishing an evolutionary relationship by series gene duplication and divergence. Furthermore, detailed knowledge of the Bs-PFK structure provides the basis for inferences concerning the structural organization of RM-PFK and the evolution of new effector sites in the enzyme tetramer.

The nearly complete amino acid sequence of RM-PFK is given in Fig. 1, together with that of Bs-PFK⁷. Strong homology

is observed among the N- and C-halves of the RM-PFK molecule and Bs-PFK. The average minimum base differences per codon⁸, calculated between the halves of the RM-PFK subunit and between each half of RM-PFK and the bacterial PFK are as follows: Bs-PFK versus N-half of RM-PFK, 0.82 (319 residues compared); Bs-PFK versus C-half of RM-PFK, 0.93 (289 residues compared); and N-half versus C-half, 1.02 (329 residues compared). The respective identities for each comparison are 44%, 34% and 32%. These numbers substantiate a significant degree of homology among all three sequences; the N-half of RM-PFK is especially homologous to the bacterial enzyme. Insertions in the RM-PFK halves are easily accommodated at the surface^{9,10}, and need not alter the basic architectural theme of Bs-PFK. Clearly, there is extensive internal homology in the RM-PFK subunit, and the mammalian enzyme arose by series gene duplication of an ancestral message related to that of Bs-PFK.

The evolution of proteins proceeds with conservation of three-dimensional structure, despite wide-ranging diversification of amino acid sequence^{11,12}. Therefore, our sequence data represent strong evidence that the N- and C-halves of RM-PFK and the Bs-PFK subunit share a similar conformation. It follows that the tertiary structure of the RM-PFK subunit will be represented by two 'super' domains, each closely resembling the structure of the Bs-PFK monomer, joined by a connecting peptide of about 30 residues (Lys 320, N-half to Ala 5, C-half; Fig. 1). The tertiary structure of Bs-PFK and the location of catalytic and effector sites in the enzyme have been described by Evans and co-workers^{9,10}, and two schematic views of subunits in the Bs-PFK tetramer as related by the x- and z-dyad axes are shown in Fig. 2. Clearly, the N- and C-termini of both pairs of subunits are close enough to be joined covalently by the length of connecting peptide available. Therefore, the halves of the RM-PFK monomer could be disposed spatially as z- or x-dyad-related pairs of subunits in Bs-PFK (Fig. 2a and b, respectively). We

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Rabbit muscle-N Half	-10	-5	1	10	20	30
<i>B. stearothermophilus</i>	AeT-H-E-E-H-A-A-R-T-L-G-V-G-K-A-I-A-V-L-T-S-G-G-D-A-Q-G-M-N-A-A-V-R-A-V-V-R-V-G-I-F-T-G-A-E-V-F-F					
Rabbit muscle-C Half			H ₂ N-M-K-R-I-G-V-L-T-S-G-G-N-S-P-G-M-N-A-A-I-R-S-V-V-R-K-A-I-Y-H-G-V-E-V-Y-G			A-V-M-N-V-G-A-P-A-A-G-M-N-A-A-V-R-S-T-V-R-I-G-L-I-Q-G-N-R-V-L-V
Rabbit muscle-N Half	40	50	60	70	80	
<i>B. stearothermophilus</i>	V-H-E-G-Y-Q-G-L-V-D-G-G-D-H-I-E-A-T-W-E-S-V-S-M-M-L-Q-L-G-G-T-V-I-G-S-A-R-C-K-D-F-R-E-E-G-R-L-R					
Rabbit muscle-C Half	V-Y-H-G-Y-A-G-L-I-A-G-M-I-E-K-L-E-V-G-D-V-G-D-I-I-H-R-G-G-T-I-L-Y-T-A-R-C-P-E-F-K-T-E-E-G-E-K-K					V-H-D-G-F-E-G-L-A-K-G-Q-I-E-E-A-G-W-S-Y-Y-V-G-G-W-T-G-Q-G-S-K-L-G-S-K-R-T-L-P-K-K-S-F-E
Rabbit muscle-N Half	90	100	110			
<i>B. stearothermophilus</i>	A-A-H-N-L-V-K-R-G-I-T-N-L-C-V-I-G-G-D-G-S-L-T-G-A-D-T-F-R-S-E-W-S-D-L-L-S-D-L-Q-K-A-G-K-I-T-A-E-E-A					
Rabbit muscle-C Half	G-I-E-Q-L-K-K-H-G-I-Q-G-L-V-V-I-G-G-D-G-S-Y-Q-G-A-K-K-L					T-E-H-G D-E-L-C
Rabbit muscle-N Half	120	130	140	150	160	
<i>B. stearothermophilus</i>	T-R-S-S-Y-L-M-I-V-G-L-V-G-S-I-D-N-D-F-C-G-T-D-M-T-I-G-T-D-S-A-L-H-R-I-T-E-I-V-D-A-I-T-T-T-A-Q-S-H-Q					
Rabbit muscle-C Half	F-P-C-V-G-V-P-G-T-I-D-N-D-I-P-G-T-D-F-T-I-G-F-D-T-A-L-N-T-V-I-D-A-I-D-K-I-R-D-T-A-T-S-H-E					I-P-F-V-I-P-A-T-V-S-N-N-V-P-G-S-D-F-S-V-G-A-D-T-A-L-N-T-I-C-T-T-C-D-R-I-K-Q-I-A-A-G-T-K
Rabbit muscle-N Half	170	180	190	200	210	
<i>B. stearothermophilus</i>	R-T-F-V-L-E-V-M-G-R-H-C-G-Y-L-A-L-V-T-S-L-S-C-G-A-D-W-V-F-I-P-E-C-P-P-D-D-N-W-E-D-H-L-C-R-R-L-S-E-T					
Rabbit muscle-C Half	R-T-Y-V-I-E-V-M-G-R-H-A-G-D-I-A-L-W-S-G-L-A-G-G-A-E-T-I-L-I-P-E-A-D-Y-D-M-N-D-V-I-A-R-L-K-R-G-H-E					R-V-F-I-I-E-T-M-G-G-Y-C-G-Y-L-A-T-M-A-G-L-A-A-G-A-D-A-A-Y-I-F-E-E-P-F-T-I-R-D-L-Q-A-N-V-E-H-L-V-Q-K
Rabbit muscle-N Half	220	230	240	250		
<i>B. stearothermophilus</i>	R-T-R-G-S-R-L-N-I-I-I-V-A-E-G-A-I-D-S-N-G-K-F-I-T-S-E-G-V-K-D-L-V-V-R-R-L-G-Y-D-T-R-V-T-V-L-G-H-V					
Rabbit muscle-C Half	R-G-K-K-H-S-I-I-I-V-A-E-G-V-G-S-G-V-D-F-G-P-Q-I-Q-E-A-T-G-F-E-T-R-V-T-V-L-G-H-V					M-K-T-T-V-K-R-G-L-V-L-R-N-E-K-C-N-E-N-Y-T-T-D-F-I-F-N-L-Y-S-E-E-G-K-G-I-F-D-S-R-K-N-V-L-G-H-M
Rabbit muscle-N Half	260	270	280	290	300	
<i>B. stearothermophilus</i>	Q-R-Q-G-T-P-S-A-F-D-R-I-L-G-S-R-M-G-V-E-A-V-M-A-L-L-E-G-T-P-D-T-P-A-C-V					
Rabbit muscle-C Half	Q-R-Q-G-S-P-T-A-F-D-R-V-L-A-S-R-L-G-A-R-A-V-E-L-L-L-E-G-E-G-G-R-C-V					Q-Q-G-G-S-P-T-P-F-D-R-N-F-A-T-K-M-G-A-K-A-M-N-W-M-A-G-E-I-E-E-S-Y-R-N-G-R-I-F-A-N-T-P-D-S-G-C-V
Rabbit muscle-N Half	310	320	330			
<i>B. stearothermophilus</i>	V-S-I-S-G-N-Q-A-V-R-L-P-L-M-E-C-V-Q-V-T-K-D-V-T-K-A-M-D-E-K-R-F-D-E-A-M-K-L-R-G-R-S-F-M-N-N-W-E-V-Y					
Rabbit muscle-C Half	G-I-Q-N-N-Q-L-V-D-H-D-I-A-E-A-L-A-N-K-H-T-I-D-Q-R-M-Y-A-L-S-K-E-L-S-I-COOH					L-G-M-R-K-R-A-L-V-F-Q-P-V-T-E-L-Q-N-Q-T-D-F-E-H-R-I-P-K-E-Q-W-W-L-K-L-R-P-I-L-K-I-L-A-K-Y-E-I-D-L-D
Rabbit muscle-N Half	340	350				
<i>B. stearothermophilus</i>	K-L-L-A-H-I-R-P-P-A-P-K-S-G-S-Y-T-V					
Rabbit muscle-C Half	T-S-E-H-A-H-L-E-H-I-S-R-K-R-S-G-E-A-T-V-COOH					

(P)

Fig. 1 The amino acid sequences of the N- and C-halves of RM-PFK compared with that of Bs-PFK. Numbering of residues is based on the Bs-PFK sequence⁷ revised by crystallographic analysis¹⁰ as follows: the tripeptide G-V-Y has been inserted after Y-35. In addition, V-43 and L-225 have each been changed to G; S-264 and A-265 have been reversed (P. R. Evans, unpublished). After residue 319, numbering is based on residues in the C-half. The Bs-PFK sequence is placed between the RM-PFK halves to emphasize homology. V-353 (N-half) is peptide-bonded to A-5 in the C-half of RM-PFK. S-348 (C-half) is the site of phosphorylation²⁰. The region enclosed in parentheses (residues 81-106, C-half) is uncertain at present. Overlaps between N-half residues 50-51, 145-146, 162-163, 261-262 and 266-267 are based on homology and have yet to be proven.

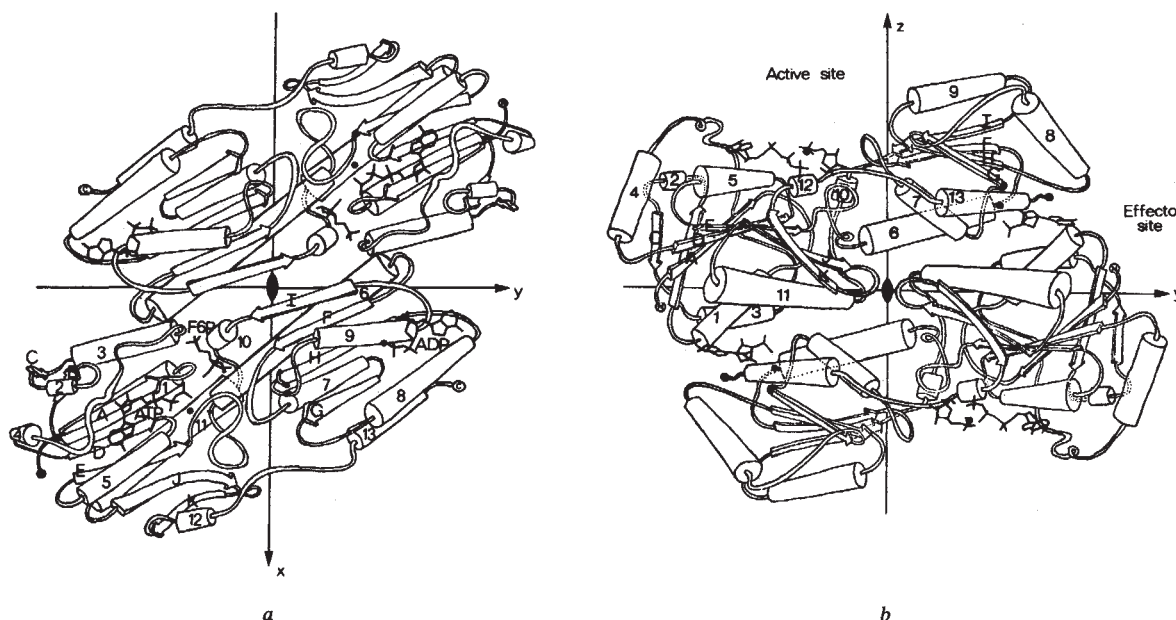


Fig. 2 Schematic views of two subunits in the Bs-PFK tetramer: *a*, viewed along the *z*-axis; *b*, viewed along the *x*-axis. In each case, the other two subunits lie behind those shown. β -Strands are indicated by arrows (A–K) and α -helices by cylinders (1–13). The substrates ATP and F6P are shown in the active site and the activator ADP in the effector site. (Taken from Evans *et al.*¹⁰)

prefer the alignment depicted in Fig. 2*b*, giving an RM-PFK subunit in which the halves are related by the *x*-dyad. This preference is based on internal helical packing interactions which would stabilize the subunit, and the fact that cooperativity in binding of fructose 6-phosphate (F6P) occurs between *z*-dyad-related subunits in Bs-PFK¹⁰. Moreover, the dimer is thought to be inactive^{13,14}, as would be predicted by disruption of the F6P binding site. Fructose biphosphate, like F6P, contributes to stabilization of the tetramer^{14,15}, and our view is that the FBP site is a mutated F6P site lying between the same *z*-dyad pair (see below). Covalent joining of the *z*-dyad subunits would create cooperative allosteric and active sites within, rather than between, subunits in RM-PFK, and we consider this mode of attachment to be less likely.

Speculation concerning the quaternary structural organization of RM-PFK is based on the structural relationship to Bs-PFK and the known D2 symmetry of the mammalian tetramer¹⁶. It is possible to bring two large RM-PFK subunits into juxtaposition to give a mammalian dimer equivalent to the Bs-PFK tetramer. Two views of such a dimer are represented by the models in Fig. 3*a*: here, the N- and C-halves of each subunit (light and dark, respectively) are joined by a connecting peptide (indicated by a helical strand of wire). The large and small lobes in each half represent the domains in Bs-PFK⁹; thus, the equivalent array of domains is preserved in our model of the RM-PFK dimer. In order to generate a tetramer having D2 symmetry, RM-PFK dimers must be brought together along the *y*-axis with all connecting peptides either on the outer or inner faces. Our preference for placing all the connecting peptides at the dimer interface (Fig. 3*b*) is supported by results from limit proteolysis. Treatment of native RM-PFK with subtilisin yields an inactive tetramer in which the N-terminal 50–60 residues have been removed¹⁷, suggesting that the N-half of each subunit is pointing outwards and is susceptible to proteolysis, and that the intersubunit forces maintaining the tetramer are still intact. These forces may well involve interactions among the connecting peptides. We have also shown that of several enzymes tested, only trypsin and endoproteinase Lys-C cleave in the connecting peptide region. This argues in favour of our proposed shielded and symmetrical arrangement of these segments in the tetramer.

Crystallographic analysis of Bs-PFK has revealed the active site regions for binding the substrates F6P and ATP, and an allosteric site for the activator, ADP. These sites were designated

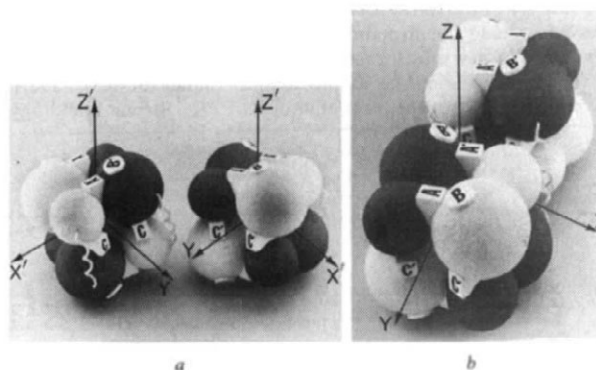


Fig. 3 Models indicating our proposed structural organization of RM-PFK. Two views of the dimer and the tetramer are shown in *a* and *b*, respectively. The N- and C-halves of each subunit are light and dark, respectively, and connecting peptides are represented by helical strands of wire. Each side-by-side dimer is equivalent to the Bs-PFK tetramer⁹, and the dimers are brought together to give a tetramer with D2 symmetry (*b*) in which all four connecting peptides are localized at the interface. The models also predict the location of sites for binding F6P (A), FBP (A'), ATP substrate (B), ADP activator (C), and ATP inhibitor (C'). Only one of the four C sites is visible in *b*; the other three are in corresponding positions at the interface. The remaining two C' sites are at the opposite end of the tetramer.

A, B, and C, respectively⁹. Sites which we believe have mutated from A and C are denoted A' and C' (see Fig. 3). Doubling of the Bs-PFK structure should give a RM-PFK tetramer with eight active sites and eight allosteric sites. Equilibrium binding studies have revealed, however, that there are only four catalytic sites in RM-PFK^{18,19}, and the greater number of effectors regulating the mammalian enzyme suggests that some catalytic and allosteric sites in the eukaryotic PFKs may have differentiated to provide new functionalities in these enzymes. Evidence in support of this notion derives from our comparative sequence analysis (Fig. 1). Homology in the vicinity of Asp-127 in Bs-PFK, a residue involved in F6P binding¹⁰, is clearly evident in the N-half of RM-PFK but not in the C-half, where the corresponding residue is a serine. We believe, therefore, that the F6P site (A) is conserved as part of the catalytic apparatus of RM-PFK,

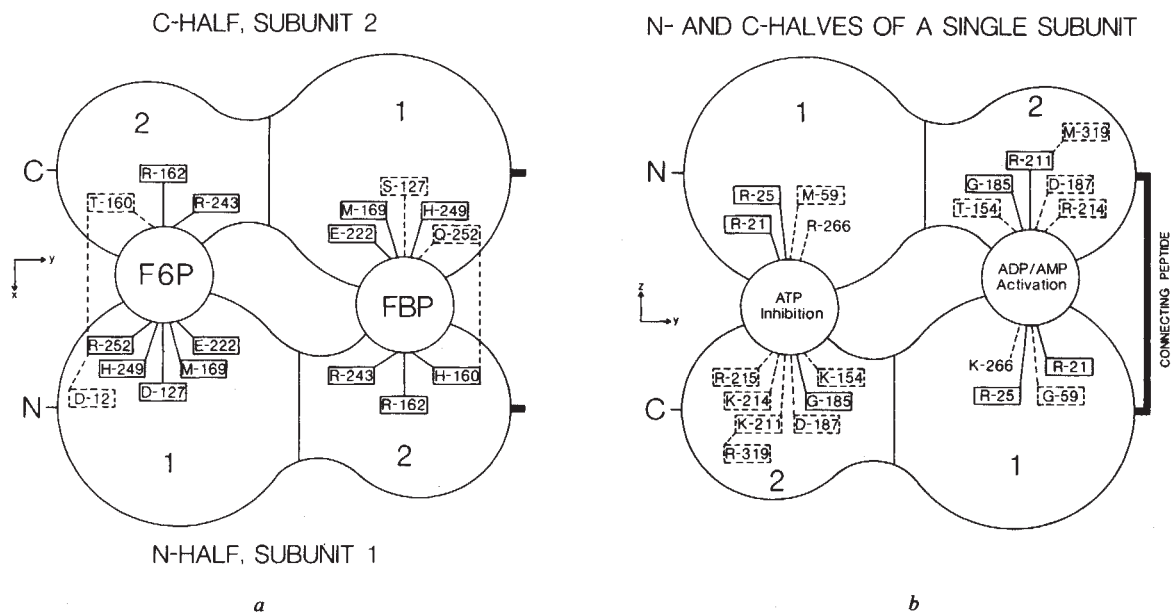


Fig. 4 Location of substrate and effector sites in RM-PFK based on crystallographic analysis of Bs-PFK and comparative sequence analysis. Residues enclosed in solid boxes are identical to those at corresponding positions in Bs-PFK; those enclosed in dotted boxes are different. *a*, View along the z-dyad axis of the N- and C-halves of two adjacent subunits which together define sites for cooperative binding of F6P (site A) and FBP (site A'). Dotted lines between residues indicate possible hydrogen bonds¹⁰. *b*, View along the x-dyad axis of the halves of a single RM-PFK subunit in which residues presumed to be involved in binding ADP activator (C site) and ATP inhibitor (C' site) are noted (compare with Fig. 3). The solid bar labelled 'connecting peptide' represents the covalent bridge between the halves related by the x-dyad axis (Figs 2b, 3). Position 266 is not boxed because its possible involvement in ligand binding was suggested by chemical modification²¹ and not by crystallography.

but that its counterpart in the C-half (A') now binds FBP (Fig. 4a). This seems reasonable as Asp 127 is probably required as a base catalyst acting to increase the nucleophilicity of the O-1 hydroxyl of F6P for attack on the γ -phosphate of ATP. Its presence would cause electrostatic repulsion of FBP; replacement of Asp 127 by Ser in the new A' site would remove this negative charge and provide more room for binding of the fructose 1,6- and 2,6-bisphosphates which are known to compete for a common site¹⁵. Thus, it is reasonable to suggest that the structural change leading to replacement of the catalytically essential Asp 127 has led to loss of a catalytic site (A) and creation of an allosteric site for FBP activation (A').

The arguments thus far account for the presence of four catalytic sites in the RM-PFK tetramer, and provide evidence as to how four new sites for binding FBP may have arisen in evolution. Furthermore, the cooperativity in binding of F6P and FBP is in keeping with our model of the tetramer where binding is between subunits (Figs 3b, 4a). The case is not so clear-cut for a possible mutation of the ADP activation site (C) to a new site for ATP inhibition (C'). Here the inferences rest more on results from limit digestion with subtilisin¹⁷ than on comparative sequence analysis. Although the subtilisin-treated RM-PFK tetramer retains the ability to bind FBP and ADP, it no longer binds ATP at the inhibition site, nor F6P. These results for a tetrameric PFK derivative having degraded N-termini are consistent with our location of F6P and FBP sites, A and A', respectively (Figs 3b, 4a). They also lend credence to the idea that the ADP activation site (C) involves residues in each subunit that are protected from the proteinase, that is, in the middle or C-terminal regions far enough removed from the N-terminal segment (Fig. 4b). Accordingly, the ATP inhibition site (C' in Fig. 3) could be a mutated ADP activation site, composed of N-terminal amino acid side chains, that is destroyed by subtilisin (Fig. 4b). We suggest, therefore, that RM-PFK has retained four of the ADP activation sites of its bacterial progenitor, but that it has gained, through evolution of the remaining four possible ADP sites, four new ATP inhibition sites per tetramer.

Thus, our model of RM-PFK predicts that the N- and C-halves of each subunit in the tetramer are equivalent in tertiary and

quaternary structural organization to the subunits in a hypothetical Bs-PFK octamer composed of two bacterial enzyme tetramers joined end-to-end along the y-axis. The A sites for F6P binding and the C' ATP inhibition sites are exposed and comprise side chains from residues in the N-terminal regions, while the A' and C sites which bind FBP and ADP activators, respectively, are made up of residues that are internal, or C-terminal in the subunits which are protected from digestion with subtilisin. At present we have no crystallographic data pertaining to the subunit organization or functional sites of RM-PFK. However, our results obtained by comparative sequence analysis, together with findings from X-ray crystallography of Bs-PFK, chemical modification studies, kinetic analysis and limit proteolysis, can be interpreted to give a unified tentative working model of the mammalian enzyme tetramer which can be addressed experimentally.

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1. Uyeda, K. *Adv. Enzym. Related Areas molec. Biol.* **48**, 193-244 (1979).
2. Kemp, R. G. & Foe, L. G. *Molec. cell. Biochem.* **57**, 147-154 (1983).
3. Paetkau, V. H., Younathan, E. S. & Lardy, H. A. *J. molec. Biol.* **33**, 721-731 (1968).
4. Coffee, C. J., Aaronson, R. P. & Frieden, C. *J. biol. Chem.* **248**, 1381-1387 (1973).
5. Emerk, K. & Frieden, C. *Archs Biochem. Biophys.* **164**, 233-240 (1974).
6. Walker, I. D., Harris, J. I., Runswick, M. J. & Hudson, P. *Eur. J. Biochem.* **68**, 255-269 (1976).
7. Kolb, E., Hudson, P. J. & Harris, J. I. *Eur. J. Biochem.* **108**, 587-597 (1980).
8. Fitch, W. M. *J. molec. Biol.* **49**, 1-14 (1970).
9. Evans, P. R. & Hudson, P. J. *Nature* **279**, 500-504 (1979).
10. Evans, P. R., Farrants, G. W. & Hudson, P. J. *Phil. Trans. R. Soc. B* **293**, 53-62 (1981).
11. Keim, P., Heinrikson, R. L. & Fitch, W. M. *J. molec. Biol.* **151**, 179-197 (1981).
12. Rossmann, M. G., Liljas, A., Branden, C. I. & Banaszak, L. J. in *The Enzymes* Vol. 11 (ed. Boyer, P. D.) 61-102 (Academic, New York, 1975).
13. Paetkau, V. & Lardy, H. A. *J. biol. Chem.* **242**, 2035-2042 (1967).
14. Lad, P. M., Hill, D. E. & Hammes, G. G. *Biochemistry* **12**, 4303-4309 (1973).
15. Foe, L. G., Latshaw, S. P. & Kemp, R. G. *Biochemistry* **22**, 4601-4606 (1983).
16. Foe, L. G. & Trujillo, J. L. *J. biol. Chem.* **255**, 10537-10541 (1980).
17. Gottschalk, M. E., Latshaw, S. P. & Kemp, R. G. *Biochemistry* **22**, 1082-1087 (1983).
18. Kemp, R. G. & Krebs, E. G. *Biochemistry* **6**, 423-434 (1967).
19. Hill, D. E. & Hammes, G. G. *Biochemistry* **14**, 203-213 (1975).
20. Kemp, R. G., Foe, L. G., Latshaw, S. P., Poorman, R. A. & Heinrikson, R. L. *J. biol. Chem.* **256**, 7282-7286 (1981).
21. Weng, L., Heinrikson, R. L. & Mansour, T. E. *J. biol. Chem.* **255**, 1492-1496 (1980).

Successive incorporation of force-generating units in the bacterial rotary motor

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Mot mutants of *Escherichia coli* are paralysed: their flagella appear to be intact but do not rotate¹. The *motA* and *motB* gene products are found in the cytoplasmic membrane²; they do not co-purify with flagellar basal bodies isolated in neutral detergents¹. Silverman *et al.* found that *mot* mutants could be 'resurrected' through protein synthesis directed by λ transducing phages carrying the wild-type genes³. Here, we have studied this activation at the level of a single flagellar motor. Cells of a *motB* strain carrying plasmids in which transcription of the wild-type *motB* gene was controlled by the *lac* promoter were tethered to a glass surface by a single flagellum. These cells began to spin within several minutes after the addition of a *lac* inducer, and their rotational speed changed in a series of equally spaced steps. As many as 7 steps were seen in individual cells and, from the final speeds attained, as many as 16 steps could be inferred. These experiments show that each flagellar motor contains several independent force-generating units comprised, at least in part, of *motB* protein.

Cells of a *motB* inducible strain were tethered to a coverslip, exposed to a *lac* inducer, and viewed with a microscope linked to a video recorder. The rotational behaviour of one such cell is analysed in Fig. 1. The cell remained in roughly one position for 16.3 min following the addition of inducer, executing brownian motion with a fixed mean orientation about its point of attachment. Then it suddenly began to spin, gaining speed over a period of ~ 17 min before levelling off at a constant speed. Abrupt changes in rotation rate were evident, particularly at the slowest speeds. Most cells in the population of 32 studied started to spin between 5 and 8 min from the time of induction; these latencies ranged from as little as 3 min to over 25 min. The time spent at the lowest speed was highly variable, ranging from a few seconds to a few minutes. The cell of Fig. 1 started spinning counterclockwise (as viewed with the cell body in front of the tethering surface, the direction corresponding to smooth swimming⁴), and, as the rotation rate increased, spun alternately clockwise (CW) and counterclockwise (CCW). The directional bias (the probability of spinning CCW) of the cell was unity at the lowest speed, but it fell to a lower value soon afterwards and remained relatively constant for the remainder of the experiment. The reversal rate was zero at the lowest speed, then increased dramatically during the next few minutes and levelled off long before the cell reached its maximum speed. The asymptotic values for directional bias and reversal rate were similar to those found for wild-type cells^{5,6}. Cells rarely changed direction at the slowest speed: only two of the cells studied showed brief CW intervals at this level, but all reversed frequently at higher levels. At any given level, cells that reversed spun CW or CCW at the same speed.

Figures 2, 3 document stepwise changes in rotation rate for two more cells. A rapid doubling of rotation rate was characteristic of the first transition: for 15 cells in which this transition was clearly resolved, the mean speed ratio ($\pm$ s.e.) was 2.05 ± 0.08 . Note that the cell of Fig. 3 underwent transitions that either increased or decreased the speed by one level, spinning at four different levels over the interval shown. Evidently, the incorporation of functional force-generating units is not irreversible.

By arbitrarily assigning the initial rotation rate of a cell to level 1, the next higher rate to level 2, etc., a set of levels was derived for each cell. Figure 4 plots mean rotation rate against level number for four representative cells in which multiple levels were observed. As many as seven distinct levels (excluding

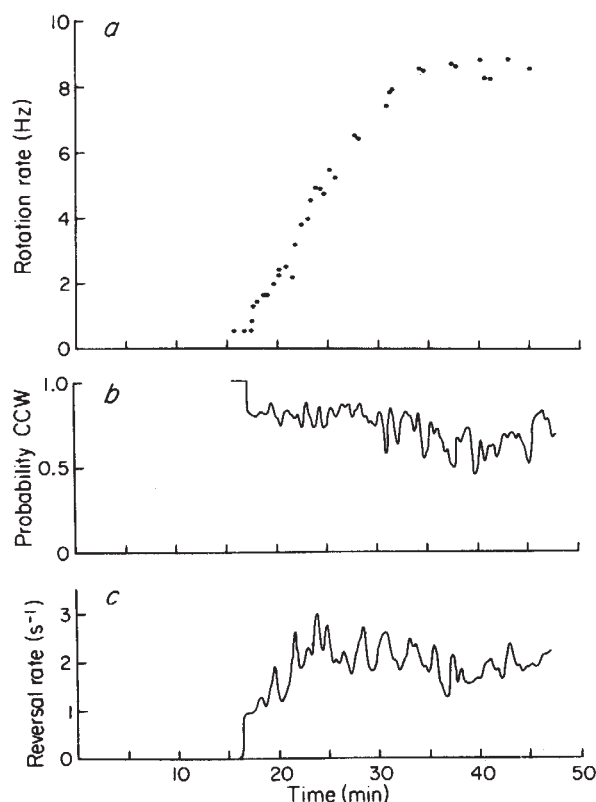


Fig. 1 Rotational behaviour of a single tethered cell, following initiation of transcription of the wild-type *motB* gene at time $t = 0$. *a*, Time course for induction of rotation; *b*, directional bias; *c*, reversal rate. The points in *a* show the average rotation rate computed from 30–300 revolutions registered by computer. Directional bias and reversal rate were determined as described previously^{5,6}. The cell began to spin at $t = 16.3$ min at 0.53 ± 0.03 Hz (mean $\pm$ s.e.m. for 33 revolutions). It levelled off at $t = 33$ min at 8.57 ± 0.07 Hz (mean $\pm$ s.e.m. for last nine data points).

Methods: Cells of strain HB154 (see below) were grown in minimal medium, washed and tethered in motility medium, as described previously⁶, except that ampicillin ($50 \mu\text{g ml}^{-1}$) and tetracycline ($10 \mu\text{g ml}^{-1}$) were present in all media. At $t = 0$, the motility medium was replaced by a minimal medium containing glycerol and essential amino acids¹⁹, as well as 1 mM isopropyl- β -D-thiogalactopyranoside (IPTG)²⁰. In subsequent experiments, the cells were exposed to this medium (without IPTG) 10 min before the addition of the inducer; this appeared to increase the number of cells that responded without changing the kinetics of their induction. The flow cell was mounted on the temperature-controlled stage (32°C) of an inverse phase-contrast microscope (Nikon Optiphot with a BM $\times 40$ objective) linked to a video camera (Ikegami ITC-47; 2:1 interlace) and recorder (Panasonic NV-8950, VHS format). A digital time display (accurate to 1/60 s, a single video frame) was superposed on the video image²¹. The speed analysis of the videotaped records is described in Fig. 2 legend. Construction of *motB*-inducible strain HB154 began with plasmid pGM1, a pBR322 derivative carrying ampicillin resistance and a restriction fragment of the *mocha* operon²² fused to *lacZ*, originally produced to direct *motB* overproduction¹⁸. The fragment contained the C-terminus of the *motA* gene and a complete copy of the *motB* gene. A λ gt1-pGM1 hybrid phage was made by cutting the plasmid and the phage at their unique *EcoRI* sites, ligating the mixture, and using the resultant constructs to infect *E. coli* strain RP3087 (an AW405 derivative²³ prepared by J. S. Parkinson carrying the *motB*⁵⁸⁰ allele¹ and deleted for λ -att) in the presence of ampicillin. An ampicillin-resistant transductant was selected which was lysogenic for λ ; this cell presumably maintains the phage in a plasmid state using the pBR322 origin of replication. An F' element carrying *lacI*^Q was then crossed into this strain to repress the *lac* promoter²⁴. The hybrid phage copy number was reduced by adding a second plasmid, pMB9, bearing tetracycline resistance²⁵, and the transformed strain, designated HB154, was maintained under double antibiotic selection. This strain was immotile in the absence of a *lac* inducer; when tethered, less than 3% of cells showed any evidence of rotation.

level 0) were resolved using the video technique. For objects the size of bacteria, the angular rotation rate is strictly proportional to the applied torque; therefore, the linearity of these plots implies that the steps in torque associated with transitions between levels are equal. Evidently, each mot protein, or protein complex, contributes the same increment of torque to the flagellar motor, at least for the incorporation of up to seven force-

generating units. Unfortunately, most cells in which multiple levels could be measured came off the glass or became stuck to it, prematurely terminating the experiment at speeds ranging from ~ 2 to 8 Hz.

The cell of Fig. 1 was an exception; the rotation rate of the first level was measured accurately, and the cell continued to spin until its speed reached a maximum value. In this case, the ratio of the final to the initial speed (16.2 ± 0.8) provides an estimate of the total number of force-generating units in the intact motor. If the efficiency of the flagellar motor remained constant over the range of speeds covered (as argued below) and all the force-generating units continued to operate independently, the motor in this cell contained about 16 such units. We also compared the mean rotation rate at level 1 for the population of cells studied here (0.76 ± 0.08 Hz; mean $\pm$ s.e.m. for 14 cells) with that of fully energized wild-type cells used in a previous study⁷ (11.20 ± 0.46 Hz; mean $\pm$ s.e.m. for 33 cells). The ratio of these means ($\pm$ s.e.) is 14.7 ± 1.7 , again suggesting that each flagellar motor could contain up to 16 independent force-generating units. Sixteenfold rotational symmetry has been found for an M-ring of the *E. coli* flagellar motor⁸. This is also the number (usually 15, sometimes 14 or 16) of membrane particles ('studs') seen at the periphery of annular depressions in freeze-fracture electron micrographs of the cytoplasmic membrane at the flagellated poles of another bacterium, *Aquaspirillum serpens*⁹.

The data of Fig. 4 do not indicate the existence of a rotational threshold: each curve extrapolates to the origin. It is possible

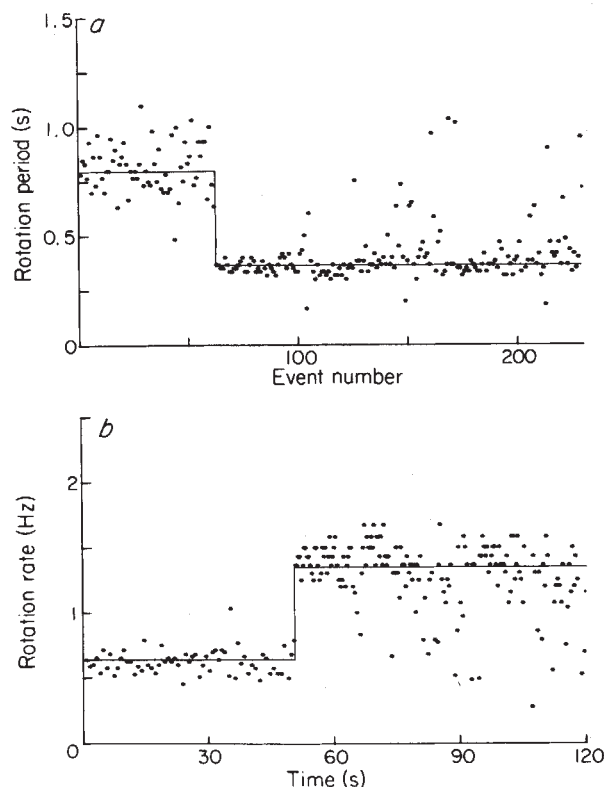


Fig. 2 Stepwise doubling in rotation rate of a tethered cell induced for motB synthesis. *a*, The first 280 rotation periods, plotted sequentially; *b*, rotation rate as a function of time computed from these periods. The solid lines represent the mean periods or rates for different intervals; see below. The cell started to spin 6.9 min after addition of inducer and doubled its rotation rate 52 s later, jumping from 1.28 ± 0.08 Hz (mean $\pm$ s.e.m. for 61 revolutions) to 2.67 ± 0.10 Hz (mean $\pm$ s.e.m. for 166 revolutions), a factor of 2.09 ± 0.15 . The transition was completed in less than four video frames (67 ms), our limit of resolution.

Methods: The rotation periods were measured with a device linked to a computer (Apple II⁺, containing a California Computer Systems 7440A Programmable Timer card connected to custom-built electronics) that generated a pulse whenever the image of a particular cell crossed a video cursor. The intervals between successive passages were stored in arrays for subsequent analysis. All of these data points are shown in the figure. The instantaneous rotation rate was computed from the reciprocal of each rotation period. Note that the points at large rates tend to fall on 'layer lines'—this reflects the fact that video images are recorded at 60 frames s^{-1} ; the time intervals are constrained to be multiples of 16.7 ms. This limitation is significant when the rates are high. As the cells often changed direction, some intervals did not correspond to complete revolutions of the cell body. These intervals were shorter than adjacent intervals if the image changed direction within half a cycle after crossing the cursor; otherwise they were longer. Also, cells had a tendency to stick to the glass; when this happened, the measured period was abnormally long. An interactive graphics program allowed the operator to identify and eliminate most of these events from calculations of mean periods or rates. The standard error for each mean was obtained from the root mean square deviation from the mean of the edited distribution; this deviation was divided by the square root of the number of rotation periods and then adjusted upwards to compensate for the truncation due to editing (assuming that the parent distribution was gaussian).

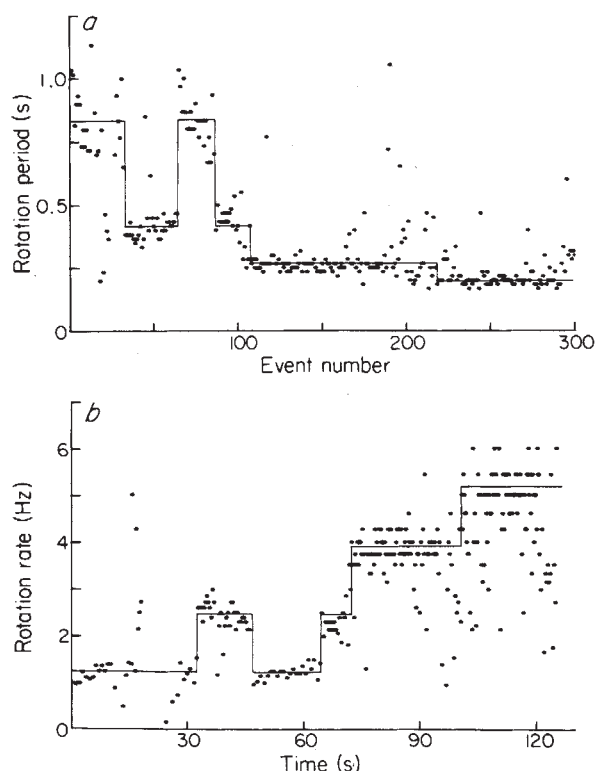


Fig. 3 Stepwise changes in rotation rate of another tethered cell induced for motB synthesis. *a*, The first 300 rotation periods, plotted sequentially; *b*, rotation rate as a function of time computed from these periods. The solid lines represent the mean periods or rates for different intervals, computed as described in Fig. 2 legend. The cell started to spin 5.9 min after the addition of inducer. Mean rotation periods tended to decrease in a harmonic series (as $1/n$, with n an integer); the corresponding rotation rates increased in an arithmetic series (as n). For large n , transitions between rotational periods of different mean amplitude became increasingly difficult to discern; as a result, the scatter in rotation rates became relatively more severe.

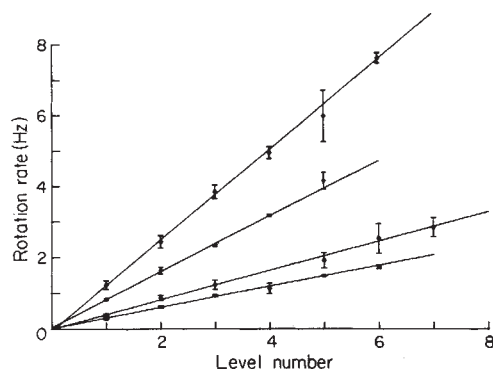


Fig. 4 Dependence of rotation rate on level number for four different cells. The vertical bars show the s.e.m., computed as described in Fig. 2 legend, and the solid lines are weighted least-squares linear fits, not constrained to pass through the origin. The different slopes reflect the variations in rotational drag from cell to cell (see text).

that the first transition that we have observed is not between levels 0 and 1, but rather between higher levels, say, 1 and 2. The possibility that a fully energized cell has no threshold is ruled out by the observation that the mean position of a paralysed cell (or flagellar filament¹⁰) remains fixed before the onset of rotation. It could be that the barrier to rotation in a fully energized cell is abolished by the introduction of functional mot protein. This question can be resolved by measuring speed as a function of protonmotive force in cells having different fixed numbers of force-generating units.

Given the increment in rotation rate from level to level, it is possible to infer the torque generated by a single force-generating unit. The constant of proportionality relating the torque generated by the motor to the rotation rate of the cell is the viscous drag coefficient, which depends only on the geometry of the cell, its position relative to the tethering surface, and the viscosity of the external medium¹¹. Drag coefficients were estimated by assuming that cells behave as cylinders with hemispherical endcaps, rotating about an axis through the centre perpendicular to the long axis¹². We neglected the interaction with the tethering surface, which is small¹³, and the fact that cells are often tethered towards one end. The latter effect can increase the viscous drag by more than a factor of two¹³, thus the drags calculated here represent an underestimate. The increments in torque from level to level for different cells appeared to be distributed normally with a mean ($\pm$ s.d.) of $2.7 \pm 0.8 \times 10^{-12}$ dyn cm (11 cells). This value may be considered an approximate lower bound for the torque associated with a single force-generating unit; the torque is approximately the same for all units in all cells.

Given this result, it is possible to calculate the number of protons passing through a force-generating unit in one revolution. The energy balance for the motor can be written as $2\pi M = \epsilon N e \Delta p$, where M is the torque, e is the electron charge, Δp the protonmotive force, N the total number of protons per revolution passing through the motor, and ϵ the motor efficiency, which could vary, for example, with angular velocity or protonmotive force. The left-hand term is the work done per revolution in driving the cell around. The right-hand term is the total work available from the proton gradient times the efficiency. Experimental evidence that the flagellar motor runs at constant torque at low speeds^{14,15} implies, for these motors, that the product ϵN is constant. For any tightly coupled motor model, in which the passage of protons through the cell membrane is obligatorily coupled to rotary motion, N is constant. Given the experimental evidence for constant torque, this implies that ϵ is constant. Furthermore, if the motor in such a model is non-dissipative, that is, if it functions reversibly with no energy going into heat, then the efficiency will be unity.

A model of this kind has been proposed in which force is

generated by the interaction of n proton channel complexes (independent force generators) situated around the periphery of a disk bearing m proton-binding sites¹⁶. The number of protons passing through a single channel complex in one revolution is m . Assuming unit efficiency, the torque generated by this channel complex can be written as $M = me\Delta p/2\pi$, where it is assumed that the efficiency is one. Given this torque and the protonmotive force, with $M \geq 2.7 \times 10^{-12}$ dyn cm and $\Delta p = 162$ mV (ref. 17), we estimate m to be ≈ 65 . The total number of protons passing through a motor with n channel complexes in one revolution is $N = nm$. Assuming that the full complement of force generators is 16, N is of the order $16 \times 65 = 1,040$, in close agreement with an earlier estimate based on similar assumptions of tight coupling and zero dissipation¹¹.

The *motB* gene product is a peptide of molecular weight $\sim 39,000$ which is known to assemble in the cytoplasmic membrane without proteolytic cleavage; this mode of insertion has been confirmed in minicell preparations which are unable to synthesize any other flagellar proteins¹⁸. Here we have found that the rotation rate of tethered, paralysed cells increased in a stepwise fashion concomitant with *motB* synthesis. The simplest explanation for this result is that the wild-type *motB* peptides diffuse to the vicinity of the flagellar motor and act there, either alone or in conjunction with other components (for example, *motA*), as independent force-generating units. We cannot exclude the possibility, however, that the *motB* protein activates some other component that, in turn, incorporates as a force-generating unit. Since the rotation rate occasionally decreased in stepwise fashion, incorporation is reversible. The chromosome of the strain that we used carried a point mutation in *motB*; therefore, defective copies of *motB* protein might have been present before induction. In that event, the addition of functional force-generating units could have involved displacement rather than simple incorporation. As many as 16 force-generating units can be accommodated—it is attractive to suppose that these are transmembrane particles comprised of *motA* and *motB* protein^{15,16}.

We thank Chris Kaiser for the construction of strain HB154, which made this work possible; other strains were provided by Gail Mandel and Sandy Parkinson. We thank Felice Borisy for technical assistance; Markus Meister and Mel Simon for helpful discussions; and Shahid Khan, Jeff Segall, Pat Conley and Bob Smyth for their comments on the manuscript. This work was supported by US NSF grant PCM-8215126. S.M.B. acknowledges support of a Proctor and Gamble Fellowship and from the National Research Service Award Program of the NIH.

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- Armstrong, J. B. & Adler, J. *Genetics* **61**, 61–66 (1969).
- Silverman, M., Matsumura, P. & Simon, M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3126–3130 (1976).
- Ridgway, H. F., Silverman, M. & Simon, M. I. *J. Bact.* **132**, 657–665 (1977).
- Larsen, S. H., Reader, R. W., Kort, E. N., Tso, W.-W. & Adler, J. *Nature* **249**, 73–77 (1974).
- Block, S. M., Segall, J. E. & Berg, H. C. *Cell* **31**, 215–226 (1982).
- Block, S. M., Segall, J. E. & Berg, H. C. *J. Bact.* **154**, 312–323 (1983).
- Segall, J. E., Manson, M. D. & Berg, H. C. *Nature* **296**, 855–857 (1982).
- DePamphilis, M. L. & Adler, J. *J. Bact.* **105**, 384–395 (1971).
- Coulton, J. W. & Murray, R. G. E. *J. Bact.* **136**, 1037–1049 (1978).
- Ishihara, A., Yamaguchi, S. & Hotani, H. *J. Bact.* **145**, 1082–1084 (1981).
- Berg, H. C. *Nature* **249**, 77–79 (1974).
- Garcia de la Torre, J. & Bloomfield, V. A. *Q. Rev. Biophys.* **14**, 81–139 (1981).
- Berg, H. C. in *Cell Motility* Vol. 3, Bk C (eds Goldman, R., Pollard, T. & Rosenbaum, J.) 47–56 (Cold Spring Harbor Laboratory, New York, 1976).
- Berg, H. C. & Turner, L. *Nature* **278**, 349–351 (1979).
- Manson, M. D., Tedesco, P. M. & Berg, H. C. *J. molec. Biol.* **138**, 541–561 (1980).
- Berg, H. C. & Khan, S. in *Mobility and Recognition in Cell Biology* (eds Sund. H. & Veeger, C.) 486–497 (Walter de Gruyter, Berlin, 1983).
- Slonczewski, J. L., Rosen, B. P., Alger, J. R. & Macnab, R. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6271–6275 (1981).
- Boyd, A., Mandel, G., & Simon, M. I. *Symp. Soc. exp. Biol.* **35**, 123–137 (1982).
- Hazelbauer, G. L., Mesibov, R. E. & Adler, J. *Proc. natn. Acad. Sci. U.S.A.* **64**, 1300–1307 (1969).
- Gilbert, W. & Müller-Hill, B. *Proc. natn. Acad. Sci. U.S.A.* **56**, 1891–1895 (1966).
- Ishihara, A., Segall, J. E., Block, S. M. & Berg, H. C. *J. Bact.* **155**, 228–237 (1983).
- Silverman, M. & Simon, M. *Nature* **264**, 577–580 (1976).
- Armstrong, J. B., Adler, J. & Dahl, M. M. *J. Bact.* **93**, 390–398 (1967).
- Müller-Hill, B., Crapo, L. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **59**, 1259–1263 (1968).
- Rodriguez, R. L., Bolivar, F., Goodman, H. M., Boyer, H. W. & Betlach, M. in *Molecular Mechanisms in the Control of Gene Expression* (eds Nierlich, D. P., Rutter, W. J. & Fox, C. F.) 471–495 (Academic, New York, 1976).

Inflationary cosmology

Gary Steigman

The Very Early Universe.

Edited by G.W. Gibbons, S.W. Hawking and S.T.C. Siklos.
Cambridge University Press: 1983. Pp.480. £25, \$49.50.

THE "standard" hot big bang model provides a good framework for understanding the early evolution and present structure of the Universe. The Robertson-Walker-Friedman (RWF) models are isotropic, homogeneous solutions to Einstein's gravitational equations in the presence of matter (nucleons) and radiation (microwave photons). Penzias and Wilson's discovery of the cosmic background radiation provided dramatic confirmation that these simple models could describe the early history of the Universe, and ushered in the "modern" era in the study of cosmology. The blackbody spectrum of the microwave background is established by electromagnetic interactions during the early evolution of the Universe and preserved during its later evolution by the homogeneity and isotropy of the RWF models. Furthermore, nuclear and weak interactions occurring during the first few minutes in the evolution of the Universe lead to the synthesis of the light elements, the abundances of which are observed to be in excellent agreement with the predictions of primordial nucleosynthesis in the standard (RWF) model. As for the more recent evolution, small deviations from the perfect homogeneity of the RWF models grow with time and may account for the observed large-scale structure of the Universe (galaxies, clusters, voids and so on).

The simplest hot big bang model has proved to be remarkably efficacious; to ignore its successes would be churlish. Nonetheless there are problems. Many questions and puzzles are neither answered nor addressed within the context of the standard model. For example, the "Planck time" $t_p \approx (G\hbar/c^3)^{1/2} \approx 10^{-43}$ s is a "natural" timescale for cosmology. The present Universe is very old ($t_0 \approx 10^{10}$ yr $\approx 10^{60} t_p$) and yet very young in the sense that the spatial curvature is not yet dominant; our Universe is very "flat". The response of the standard model to the age-flatness puzzle is to appeal to initial conditions: the Universe is the way it is because it was the way it was.

A different problem is posed by the matter content of the Universe. The observed Universe consists overwhelmingly of matter; antimatter is absent. Why? Early on there must have been a tiny excess of matter over antimatter so, when nucleon-antinucleon annihilation ceased, a trace amount of relic nucleons survived. The present ratio of nucleons to photons (η) is a measure of the requisite excess: $10^{-10} \leq \eta \leq 10^{-9}$. Within the standard model

there is no explanation for the magnitude of η (or even why it should not be zero); η is the size it is because it was the size it was.

Even the observed large-scale homogeneity, a key ingredient of the standard model, is a serious puzzle. The finite speed of light guarantees the existence of particle horizons so that the various parts of the Universe from which the microwave radiation originates were never in causal contact. Why, then, is the flux observed to be so very uniform? Why, indeed, is the large-scale Universe so very homogeneous?

There are still further questions. Why is the cosmological constant so small? Is it zero? What of the singularity of the RWF models? The list goes on, but all is not darkness and despair. Less than a decade ago the tide of a revolution in cosmology began to swell. Today it has risen to a flood. Its source was high-energy physics. The successful unification of the electromagnetic and weak interactions led to the predictions of new phenomena such as neutral current interactions; the gauge theory of the strong interaction predicted especially simple behaviour at high energy (asymptotic freedom); Grand- and Super-Unified theories were proposed predicting new phenomena and new particles, often at energies far beyond or rates far below those attainable in the laboratory. Some cosmologists and a few particle physicists quickly appreciated that the early Universe was the ultimate accelerator — the particle physics laboratory of last resort. That particle physics might provide the key to solving various cosmological puzzles in the early evolution of the Universe was much less obvious. Yet the relationship between particle physics and cosmology which has blossomed in recent years, while not entirely trouble free, has been a remarkably fecund affair.

For example, a solution to the asymmetry puzzle is provided by Grand Unified Theories (GUTs) in the context of the very early evolution of the Universe. In most GUTs, baryon number is not conserved and charge (C) and charge-parity (CP) conservation are violated. As Sakharov noted in his recipe for generating a baryon asymmetry, these are two of the three required ingredients. The third, deviation from equilibrium, is provided by the expansion of the Universe. Although there are too many free parameters in the GUTs to permit a calculation of the nucleon to photon ratio, η , from first principles, it is nonetheless clear that a framework for this calculation now exists.

No longer need we appeal to initial conditions: η is what it is because of baryon number and C and CP violating interactions which occurred during the very early evolution of the Universe. The universal baryon asymmetry and the instability of the proton are two sides of the same coin.

Lest we be lulled into a false sense of security, it must be said that the same GUTs which solve the asymmetry puzzle, predict the existence of superheavy ($\sim 10^{16}$ GeV) magnetic monopoles. In the standard model these monopoles are produced during the same epochs in which the baryon asymmetry is generated and they survive in abundances comparable to that of the nucleons. The enormous mass density in the relic monopoles would have driven the Universe to expand so rapidly that today, when the blackbody radiation is at a temperature of 3K, the Universe would be only $\sim 10^3$ yr old! (It is often stated, incorrectly, that relic monopoles "close" the Universe. The curvature neither knows nor cares about the stuff of the Universe.) So, GUTs and cosmology solve the baryon asymmetry puzzle but present us with a new problem — relic monopoles; failure snatched from the jaws of success!

With the monopole problem as one of its catalysts, there then occurred a revolution within the revolution. The cosmological constant emerged from the closet to play a significant role during the very early evolution of the Universe. As Zeldovich and others have noted, the cosmological constant (Λ) may be regarded as the energy density of the vacuum ($\Lambda \equiv 8\pi G\rho_v$). Although Λ is small today ($\Lambda < H_0^2 \rho_v < 10^{-4} \text{ eV cm}^{-3}$), the energy density of the vacuum would have been enormous during the very early evolution of the Universe ($\rho_v \sim 10^{110} - 10^{126} \text{ eV cm}^{-3}$). K. Sato and A. Guth realized that the vacuum energy density would drive the early evolution of the Universe and Guth appreciated that the "inflationary" exponential expansion would, in one fell swoop, solve a myriad of cosmological conundrums. Cosmology hasn't been the same since.

In the summer of 1982, Stephen Hawking, with the support of the Nuffield Foundation, organized what turned out to be a very productive workshop in Cambridge, UK, on "The Very Early Universe". Truly international in character — the participants came from Great Britain, continental Europe, the United States and the Soviet Union — the meeting covered topics as diverse as quantum gravity and galaxy formation, monopoles, strings and massive neutrinos, photinos and axions, supersymmetry, GUTs and the cosmological constant. Not surprisingly, the "new" inflationary models of Linde and of Albrecht and Steinhardt were the centre of interest. And rightly so, for, if it could be implemented, new inflation would solve the age, flatness,

homogeneity and monopole problems. Furthermore, the reheating after the inflationary epoch would then account for the enormous entropy ($\geq 10^{87}$ photons in the observable Universe) as well as the large entropy per baryon ($\eta^{-1} \geq 10^9$ photons per nucleon) of the Universe. What is more, new inflation predicts perturbations of the spectral form first suggested by Harrison (and, therefore, now known as the Zeldovich spectrum!). The workshop was particularly noteworthy in that the participants arrived emboldened by recent successes and left with further successes and new problems with which to wrestle. The struggle still continues.

This book, a record of the workshop, provides a window on the excitement of the meeting itself. Virtually all the articles are interesting, comprehensive and well written. Several are, and will remain, valuable reviews of an entire subfield of cosmology or particle physics. Although some are highly technical, most can be tackled by the knowledgeable graduate student; cosmologists will find the particle physics articles comprehensible and vice versa. Especially worth reading are the introductory and summary reviews by Wilczek; the cosmology article (virtually a monograph on modern cosmology) by Rees; the primer on GUTs and Supersymmetry by Ellis; the self-contained introduction to and review of monopoles by Preskill; the inflationary articles by Guth,

Linde, Steinhardt and Hawking; and the outlines of the development of perturbations in the inflationary models by Turner, and by Lukash and Novikov. I have learned a great deal from these articles and expect to return to them frequently in my teaching and research. Conspicuous by its absence, however, is a contribution by Starobinsky who, to judge from the comments of many of the participants, played a major role in discussions at the meeting.

Stephen Hawking is owed a debt of grati-

tude for having attracted the right people to the right workshop at the right time. In *The Very Early Universe* Hawking and his co-editors, G.W. Gibbons and S.T.C. Siklos, have provided the astro-particle physics community with a valuable work of reference. □

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Accounts of energy

Colin Sweet

The Economics of Nuclear Energy.

Edited by L.G. Brookes and H. Motamen.

Chapman & Hall/Methuen: 1984.
Pp.420. £27.50, \$65.

ATTITUDES towards nuclear power in one country have always been more influenced by developments elsewhere than is the case with any other industry, with the possible exception of the defence industries. It is appropriate, therefore, that this new addition to the literature should set out to be international in scope. The 23 authors are drawn from six countries.

Despite its title, the book is not in any sense an economics text. Nor is it a straightforward exercise in applied economics. Rather, it is series of essays on different aspects of nuclear power as seen from different countries. This approach certainly has its merits, but to contribute something more to the current debate the book would have had to have been explicitly eclectic. As it is, while the authors have different ways of making their economic assessment (such that one cannot often be compared with another), the conclusion that they all arrive at, with one possibly neutral exception, is that nuclear power is the most attractive option on offer for future growth in electricity generation.

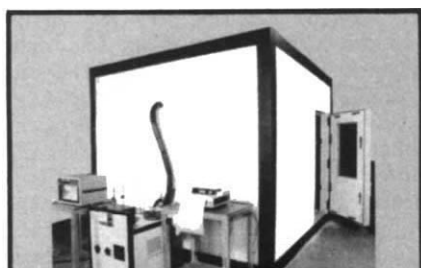
The first three chapters of the book are concerned with a systems analysis of electricity supply and are in many ways the most satisfactory. They provide a good basis for discussion of economic pricing. One point which emerges very clearly is the great complexity of the nuclear-electric system, from the mining of uranium to the delivery of the product to the consumer. And between them, these chapters — by C. Starr and O. Yu (United States), L. Gouni (France) and T. Berrie (UK) — bring out well the technical constraints of electricity systems generally. Especially for the layman this is good start. However the treatment is not without problems which, once raised, recur in the so-called economic discussions of the subject. Starr and Yu conclude that centralization of production is a logistical objective. Gouni follows by

drawing attention to the monopolistic tendency that accompanies such centralization. But like his counterpart in the UK, J. Rhys (Electricity Council), Gouni does not really face the contradiction between this reality and the assumptions of a competitive market. The result is that Rhys's chapter in particular, which is one of the few that tackles the problem of economic pricing, has an overly academic air. His conclusion that "In the long run, provided nuclear power can be established as the cheapest means, it is likely to be the dominant factor in determining the overall level of electricity prices" may be conceptually a narrow basis for making a judgement but it is about the most open-ended statement to be found in the book.

One reason why the book is methodologically weak is that it rests too much on the thinking that prevailed when nuclear economics was inseparable from propaganda of the institutionalized kind. It is not the authors' fault that their book went to press before they had time to absorb the massive amount of information put before the Sizewell "B" Public Inquiry. Nevertheless it is unfortunate. In their submissions, the Central Electricity Generating Board appear finally to have put historic cost accounting behind them for purposes of investment appraisal and to have embraced opportunity cost analytical studies. This is something which British authors have to communicate to their opposite numbers in nuclear establishments in other parts of the world. It is a pity that here the opportunity was missed.

Interestingly enough, the best comparative study of nuclear and coal-fired generation comes from D. Schmitt and H. Jung of Cologne University. They use the requirement revenue method which computes likely discounted costs of future projects against future revenues. The cost assumptions are not always convincing and the treatment of the demand side is conspicuous by its absence (on this point they could do well to read what Gouni has to say about the demand factor). They are however aware of the limitations of such economic exercises and indeed they make the observation which one would like to have seen feature more prominently in other contributions — that the future of nuclear power will not be decided by economics. "The crucial barrier is public

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acceptance" as far as Schmitt and Jung are concerned, and that means that "The expansion of nuclear energy thus becomes a problem of political decision making". They are surely right.

The least satisfactory chapters in the book are one from the United States and two others from France. The American study, by G.R. Corey, is the familiar full cost analysis which abounds in the past literature. It is replete with data that can hardly be checked and assumptions that are heroic at nearly all points. The contributions from the French Atomic Energy Authority (CEA) are the worst kind of institutional arrogance. This is a great pity, but not a surprise: the French commitment to nuclear power is not only greater than that of any other country, but their programme is beset with considerable problems of overcapacity and cost. J. Baumier and L. Duchatelle, and their colleague L. Thiriet (appropriately from the public relations department), have no wish to explore problems. Baumier and Duchatelle write about the French Fast Breeder Reactor, Super-Phénix, which from an engineering point of view may prove to be an achievement. But from the economic standpoint it is a disaster. The attempt by the authors to juggle with figures in order to show that while the first station will produce power at twice the cost of a Pressurized Water Reactor the "cost ratios will progressively approach unity" is unconvincing and ought not to be taken seriously (as I am sure it is not by Electricité de France).

Less strident but characterized by the same cover-up technique is the contribution by A. Farmer of the United Kingdom Atomic Energy Authority. The recent stinging report by the Comptroller and Auditor General on the vast sum of money that has been expended on the UK Prototype Fast Reactor at Dounreay, which nine years after completion hardly works at all, ought to raise some very real questions about project evaluation and financial monitoring of nuclear projects. A British Fast Reactor of commercial size now appears to have been abandoned but the annual research and development allocations of £100 million plus continue. For how long? With what benefits? And for whom?

As an exposition of the manner in which nuclear power has developed its own peculiar brand of economics, which must be seen of course as a part only of its rationale, this book is informative and illuminating. Yet all books on nuclear power, without exception, ought to be read critically and with a certain degree of disbelief. Because this one does not carry a health warning, it needs to be consumed with at least as much care as its predecessors. □

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The information man

Walter Gratzer

Essays of an Information Scientist, Volume 5.

By Eugene Garfield.

ISI Press, 3501 Market St, Philadelphia: 1983. Pp.848. \$25 (North America), \$28 (elsewhere).

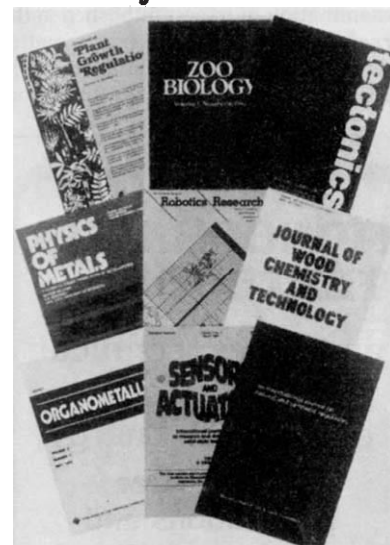
"AH, Mr Gibbon", the Duke of Gloucester is supposed to have observed, on being presented with the second volume of *The Decline and Fall of the Roman Empire*, "another damned, fat, square book. Always scribble, scribble, scribble, eh?". Likewise Eugene Garfield, whose business is the printed or processed word, and whose own output with this, his fifth volume of essays, culled from *Current Contents* and running to 848 pages, has probably by now exceeded Gibbon's life's work in the majesty of its bulk. It is true that, as he explains, in the essay entitled "How do you do it? Write all those essays, I mean", Dr Garfield has the advantage of Gibbon in that he marshals a staff of helpers who man his organization (the Institute for Scientific Information) and have titles such as Director of Communication, Manager of Bibliographic Research and Manager of Editorial Materials, not to mention two secretaries, called Sharon and Mary, their pens ever poised for dictation. All the same Dr Garfield's achievement is considerable, for he maintains a high level of readability, and from the well of statistics about science that he has sunk there gushes an inexhaustible stream of curious, interesting and sometimes outlandish information.

Dr Garfield has done more than invent *Current Contents* and the *Science Citation Index* to smooth the path of scientists: he has used the new information technology to generate a whole new human activity, which will undoubtedly become known (if it is not already) as *scientometry* or *scientometrics*. Although it had probably occurred to no one before Garfield had his first brainwave that anything of much consequence could be inferred from the references appended to every scientific publication, their function has undoubtedly undergone a surreptitious transformation. From being merely part of the *apparatus criticus*, designed to assist the reader to acquaint himself most easily with the background of the subject, references have acquired, with the emergence of "peer-review" and centralized funding, a ritual significance. They are now selected mainly to flatter, mollify or disarm. Why else, if not with a shifty eye to the impression that their bibliography might make on present or future members of a Study Section, do authors, as one of Dr Garfield's analyses has shown, cite by preference the work of their own compatriots at the expense of that from other countries? Con-

versely, scientists with undernourished egos are, as every editor knows, apt to make their dissatisfaction known if they believe their work to have received insufficient acknowledgement. As for auto-referencing (I cite Dorothy Parker), the advantage of self-praise is that it can be laid on thick and when it is most needed. Now that *Science Citation Index* ratings have even found a place in litigation, over such diverse matters as tenure or promotion and alleged maltreatment of laboratory animals, we disregard them, it seems, at our peril.

Like mercy then, citation is twice blessed (or three times if one takes account of ISI and Dr Garfield); it blesseth him that gives and him that takes. Moreover, Dr Garfield leaves us in no doubt that his league tables reflect merit in some sort. He rebuts the commonly heard argument that the best way to join that blessed host of the most cited authors — in Dr Garfield's terminology the *Nobel Class*, no less — one need only make some trifling improvement in an analytical colour reaction or devise a new buffer system for gel electrophoresis, by demonstrating that only a minority of the *Nobel Class* would be supplanted if their

New journals review



On 27 September *Nature* will publish the fourth annual review supplement devoted to science journals.

Criteria for inclusion of a journal in the 1984 issue are that:

- (i) the first number appeared, or the journal was retitled, between June 1982 and May 1983 (the second cut-off date allows at least three issues of a journal to have been published, the minimum number on which a reasonable judgement can be based);
- (ii) it is published at least three times a year;
- (iii) the main language used is English.

Publishers and learned societies are invited to send four different issues of each suitable periodical, including the first and most recent numbers (if from outside the United Kingdom, by air mail) to: The Review Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF, England. Subscription details, if possible for both 1984 and 1985, should be included.

single most cited paper were taken away. And yet, and yet . . . looking down the register of the *Nobel Class*, we see amongst the Nobel laureates and aspirants the names (I would have thought by common consent) of some of the choicest noodles in all of science, as well as some striking absences (not a Huxley in sight for example); presumably some of these operate in sparsely populated fields, or then again perhaps, as Chargaff has suggested, it is less important to be first than to be last. Garfield himself quotes a testy letter from an illustrious virologist, to the effect that his *Citation Classic* (another of Garfield's coinages) is one of his less significant accomplishments, being cited exclusively for the procedure that it contains for washing and suspending bentonite clay, which is used as a nuclease inhibitor.

All the same, whatever one's (wholly subjective) reservations, Dr Garfield's statistics afford a unique panorama of science in action, and many a diverting insight into the intimate habits of its practitioners. The refinements of authorship are particularly rewarding. We learn for example that with success comes a modicum of generosity: Nobel laureates, once the bacon is safely home, tend to yield priority to their co-workers in the listing of authors. An examination of papers published in the *Journal of Physiology*, which as a matter of editorial policy places authors' names in alphabetical order, reveals that Professors

with names such as Zweig, or those who have graduate students called Aardvark, take their business elsewhere. Indeed the citation-watchers, such as Professor Derek de Solla Price, have, it appears, been much exercised about how to apportion credit for authorship: should the first author be entitled to store up more riches in heaven than the second or the twenty-second? Should one receive equal benison for a share in a thirty-author bone-crusher of the kind common in particle physics, as for a solo effort? The experts, you will be relieved to hear, are working on it.

The scope of Dr Garfield's writings transcends the work of ISI, however. When Charles Dickens received as a contribution to the literary magazine, of which he was editor, a set of poems with the title "Orient

pearls at random strung" he sent it back with the scribbled comment, "too much string". There is, to be sure, a certain amount of string in Dr Garfield's latest anthology, but overall he gives excellent value. His organization has endowed prizes and sponsored local artists; he writes pleasantly about these enthusiasms, and his dissertations on such subjects as risk assessment, and most of all on medical matters, are at times quite transfixing. His book is worth the money just for his accounts of those three arguments against the existence of God, acne, halitosis and venereal trichonomiasis. One looks forward to more in the same vein anon. □

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Metals on the brain

Laszlo Magos

Neurobiology of the Trace Elements (in two volumes).

Edited by Ivor E. Dreosti and Richard M. Smith.

Humana: 1983. Vol.1 pp. 374, Vol.2 pp.320. Each volume \$49.50 (US), \$59.50 (elsewhere).

THE title of this work refers to trace elements, but it is not without significance that only one chapter in the two books deals with an element which is not metal or metalloid. The first volume is dedicated to *Trace Element Neurobiology and Deficiencies*, the second to *Neurotoxicology and Neuropharmacology*; neuropharmacology, however, is represented by a single contribution only.

Though there are many common targets for essential and toxic metals — for example catecholamine metabolism for copper and manganese, or hippocampus for zinc, lead and trimethyltin — market research probably indicated that interest in one volume would frequently exclude interest in the other. As evidence of this, both volumes have the same foreword, preface and conclusions. As a toxicologist I cannot agree with the assumption — I found the first volume more cohesive, comprehensive and altogether better reading. One has the impression that the editors, with experience in trace metal deficiencies, were better equipped to conduct the authors of the first volume to play in concert but did not influence the authors of the second to the same extent. Moreover the correction of inconsistencies in the chapter on manganese in Vol.2 (for example when absorption is described) would have greatly improved this contribution.

In the first volume there are two chapters on copper, three on zinc and single chapters covering iodine, iron, selenium

and cobalamine. There is very little repetition when more than one chapter is dedicated to the same metal. The authors balance well biochemistry, pathology, clinical and experimental investigations, and amalgamate diverse knowledge effortlessly, putting the main emphasis on developmental neurobiology.

The second volume has an excellent chapter on mercury, but this account is rather narrow in scope being restricted to the effects of methylmercury on the fetal brain. The hiatus is filled by a contribution on the behavioural toxicology of heavy metals, which, in addition to covering behavioural effects, concisely describes the neurotoxicity of methylmercury, metallic mercury and lead, and at least mentions arsenic and organotins. Given the effect of trimethyltin on the hippocampus, however, a chapter on these compounds should have been included. The rest of Vol.2 contains two chapters on cadmium and three on lead, with manganese, aluminium and lithium each being represented by single chapters. Though the teratogenicity of zinc deficiency is described in the first volume in detail, and the protective effect of zinc against the teratogenicity of cadmium in the second volume, it is not mentioned that cadmium inhibits the placental transport of zinc. Nevertheless the defects of the second book are relatively minor and it mainly suffers from comparison with Vol. 1.

Researchers in fields related to the neurobiology or neurotoxicology of trace metals will probably find, as I did, the reading of this not unusually expensive work to be rewarding. Beyond the information supplied, the quality of paper is excellent, the typography is clear and pleasant, and references, complete with titles, are plentiful and are arranged in alphabetical order at the end of the chapters. Both volumes are supplied with good indexes. □

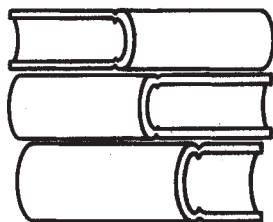
Laszlo Magos is in the Medical Research Council Toxicology Unit, Carshalton, Surrey.

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Mathematics

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Matrix Computations. By GENE H. GOLUB and CHARLES F. VAN LOAN. North Oxford Academic: 1983. Pp.476. Hbk ISBN 0-946536-007; pbk ISBN 0-946536-058. Hbk £35, pbk £19.50.

Operational Calculus, Vol. 1. 2nd Edn. By JAN MIKUSIŃSKI. Pergamon: 1983. Pp.321. ISBN 0-08-025071-8. £12, \$27.

Cosmology and Space Science

Accretion-Driven Stellar X-Ray Sources. W.H.G. LEWIN and E.P.J. VAN DEN HEUVEL (eds). Cambridge University Press: 1983. Pp.450. ISBN 0-521-24521-4. £40, \$79.50

Astronomical Phenomena for the Year 1985. HMSO: 1983. Pp.74. Pbk ISBN 0-11-886912-4. Pbk £3.

Astrophysics 1. Stars. By RICHARD BOWERS and TERRY DEEMING. Jones and Bartlett: 1984. Pp.343. ISBN 0-86720-018-9. \$37.50.

Cosmic Ecology: The View from the Outside in. By GEORGE A. STEIELSTAD. University of California Press: 1983. ISBN 0-520-04753-2. £19.20, \$24.95.

The Early Years of Radio Astronomy: Reflections Fifty Years After Jansky's Discovery. W.T. SULLIVAN III (ed.). Cambridge University Press: 1984. Pp.421. ISBN 0-521-25485-X. £25.

A Field Guide to the Stars and Planets, 2nd Edn. D.H. MENZEL and J.M. PASACHOFF. Houghton Mifflin: 1983. Pp.473. Hbk ISBN 0-395-34641-X; pbk ISBN 0-395-34835-8. Hbk \$16.95; pbk \$11.95.

The Nearby Stars and the Stellar Luminosity Function. A.G.D. PHILIP and A.R. UPGREN (eds). L. Davis Press: 1983. Pp.487. Pbk ISBN 0-960790-4-1. Hbk \$40; pbk \$30.

Planetary and Lunar Coordinates for the Years 1984-2000. HMSO: 1983. Pp.321. Pbk ISBN 0-11-886917-5. Pbk £10.

Planetary Nebulae: A Study of Late Stages of Stellar Evolution. By S.R. POTTASCH. Reidel: 1983. Pp.322. ISBN 90-277-1672-2. Dfl.115, \$43.

The Solar Granulation, 2nd Edn. By R.J. BRAY et al. Cambridge University Press: 1984. Pp.256. ISBN 0-521-24714-4. £27.50, \$54.50.

Biological Sciences

Advanced Biology. By JOHN SIMPKINS and JOHN WILLIAMS. Bell & Hyman: 1984. Pp.690. Pbk ISBN 0-7135-1387-X. Pbk £10.95.

Advanced Plant Physiology. MALCOLM B. WILKINS (ed.). Pitman: 1984. Pp.514. ISBN 0-273-01853-1. Np.

Advances in Nitrogen Fixation Research. C. VEEGER and W.E. NEWTON (eds). Martinus Nijhoff/Dr W. Junk: 1984. Pp.760. ISBN 90-247-2906-8. Dfl.235, \$87.50, £58.50.

Amino Acids, Peptides, and Proteins, Vol.14. A Review of the Literature Published during 1981. J.H. JONES (ed.). Royal Society of Chemistry: 1983. Pp.502. ISBN 0-85186-124-5. £83, \$131. Discount for Members.

Animal Pain: Perception and Alleviation. RALPH L. KITCHELL et al. (eds). American Physiological Society: 1983. Pp.221. ISBN 0-683-04625-X. Np.

Atlas of Sectional Anatomy: Head, Neck and Trunk. By PHILOMENA McGRATH and PETER MILLS. Karger: 1984. Pp.238. ISBN 3-8055-3624-0. SwFr.82, DM 98, \$49.25.

Bibliographic Atlas of Protein Spectra in the Ultra-violet and Visible Regions. DONALD M. KIRSCHENBAUM (ed.). Plenum: 1984. Pp.523. ISBN 0-306-65159-9. Np.

Biochimie Etudes Medicales et Biologiques. By JACQUES KRUH. Hermann, 293 rue Lecourbe, 75015 Paris: 1983. Pp.252. ISBN 2-7056-5959-5. Fr.120.

Biogeography and Ecology of the Pitiusic Islands. E. KUHBIER (ed.). Dr W. Junk: 1984. Pp.704. ISBN 90-6193-105-3. Dfl.335, \$145, £85.

Biology and Chemistry of Plant Trichomes. ELOY RODRIGUEZ et al. (eds). Plenum: 1984. Pp.255. ISBN 0-306-41393-0. £39.50.

The Biology of Free-Living Nematodes 2nd edition. By WARWICK L. NICHOLAS. Oxford University Press: 1984. Pp.251. ISBN 0-19-857587-4. £25.

Biomembranes and Cell Function. Annals of the New York Academy of Sciences, Vol.414. The New York Academy of Sciences: 1983. Pp.195. Hbk ISBN 0-89766-222-9; pbk ISBN 0-89766-223-7. Pbk \$40.

Biomembrane Structure and Function. Topics in Molecular and Structural Biology 4. DENNIS CHAPMAN (ed.). Macmillan London: 1984. Pp.414. ISBN 0-89573-208-4. £50.

Biosynthesis, Vol.7. A Review of the Literature Published during 1979, 1980 and 1981. R.B. HERBERT et al. (eds). Royal Society of Chemistry: 1983. Pp.223. ISBN 0-85186-553-4. £33, \$59. Discount for Members.

Brain and Pituitary Peptides II: Pulsatile Administration of Gn-RH in Hypothalamic Failure: Basic and Clinical Aspects. G. LEYENDECKER et al. (eds). Karger: 1983. Pp.187. ISBN 3-8055-3811-1. SwFr.98, DM117, \$58.75.

Cell Fusion: Gene Transfer and Transformation. Miles International Symposium Series Number 14. ROLAND F. BEERS and EDWARD G. BASSETT (eds). Raven: 1984. Pp.421. ISBN 0-89004-941-6. \$61.50.

Cellular Responses to DNA Damage. UCLA Symposia on Molecular and Cellular Biology, Vol.11. ERROL C. FREIDBERG and BRYN A. BRIDGES (eds). Alan R. Liss: 1983. Pp.768. ISBN 0-8451-2610-5. £75.

Chemical Taxonomy, Molecular Biology, and Function of Plant Lectins. Vol.138. Progress in Clinical and Biological Research. IRWIN J. GOLDSTEIN and MARILYNN E. ETZLER (eds). Alan R. Liss: 1983. Pp.298. ISBN 0-8451-0138-2. £29.

The Chimpanzees of Kibale Forest: A Field Study of Ecology and Social Structure. By MICHAEL PATRICK GHIGLIERI. Columbia University Press: 1984. ISBN 0-231-05594-3. \$45.50.

CO₂ and Plants: The Response of Plants to Rising Levels of Atmospheric Carbon Dioxide. EDGAR R. LEMON (ed.). Bowker Publishing: 1983. Pp.280. ISBN 0-86531-597-3. £21.75.

Coevolution. MATTHEW H. NITECKI (ed.). University of Chicago Press: 1983. Pp.391. Hbk ISBN 0-226-58686-3; pbk ISBN 0-226-58687-1. Hbk £25.50, pbk £13.60.

Costa Rican Natural History. DANIEL H. JANZEN (ed.). University of Chicago Press: 1984. Pp.816. Hbk ISBN 0-226-393321; pbk ISBN 0-226-393348. £25.50.

Cytochemical Bioassays: Techniques and Clinical Applications. J. CHAYEN and LUCILLE BITENSKY (eds). Butterworths: 1983. Pp.418. ISBN 0-407003444. £39.50.

Disinfection, Sterilization and Preservation. 3rd edition. By SEMOURS. BLOCK. Lea & Febiger: 1983. Pp.1053. ISBN 0-8121-0863-9. \$87.50.

Echinoid Palaeobiology. By ANDREW SMITH. Allen & Unwin: 1984. Pp.190. Hbk ISBN 0-04-563001-1; pbk ISBN 0-04-563002-X. Hbk £20, pbk £11.95.

Eleventh Symposium on Nucleic Acids Chemistry held in Tokyo, Japan November 1st-2nd, 1983. Nucleic Acids Symposium Series No.12. ANGELA E. PRITCHARD (ed.). IRL Press: 1983. Pp.224. ISBN 0-904147-53-3. Pbk £14, \$28.

Ernährung und Stoffwechsel der Pflanze. By KONRAD MENGEL. Gustav Fischer: 1984. Pp.431. ISBN 3-437-20307-X. DM48.

Evolution and the Genetics of Populations. S.D. JAYAKAR and LAURA ZONTA (eds). Università Di Pravia, via Loredan 10, 35100 Padova, Italy: 1984. Pp.162. \$15.

Evolutionary Ecology of Neotropical Freshwater Fishes. THOMAS M. ZARET (ed.). Dr W. Junk: 1984. Pp.173. ISBN 90-6193-823-6. Dfl.150, \$56, £37.

Excitotoxins. K. FUXE et al. (eds). Macmillan Press, London: 1984. Pp.362. ISBN 0-333-36152-0. £40.

Five New World Primates: A Study in Comparative Ecology. By JOHN TERBORGH. Princeton University Press: 1983. Pp.260. Hbk ISBN 0-691-08337-1 pbk ISBN 0-691-08338-X. Hbk \$40, pbk \$13.50.

Frontiers in Neuroendocrinology, Vol.8. LUCIANO MARTINI and WILLIAM F. GANONG (eds). Raven Press: 1984. Pp.324. ISBN 0-89004-293-4. \$57.50.

Gene Expression. UCLA Symposia on Molecular and Cellular Biology New Series, Vol.8. DEAN H. HAMER and MARTIN J. ROSENBERG (eds). Alan R. Liss: 1983. Pp.588. ISBN 0-8451-2607-5. Np.

Hypoxia, Exercise, and Altitude. Proceedings of the Third Banff International Hypoxia Symposium. Progress in Clinical and Biological Research, Vol. 136. JOHN R. SUTTON et al. (eds). Alan R. Liss: 1983. Pp.516. ISBN 0-8451-0136-6. £63.

Inorganic Plant Nutrition. Encyclopedia of Plant Physiology Vols 15A and 15B. A. LAUCHLI and R.L. BIELESKI (eds). Springer-Verlag: 1983. Pp.449. ISBN 3-540-12103-X/0-387-12103-X. DM 338, \$131.20.

Insulin-Like Growth Factors Somatomedins: Basic Chemistry, Biology, Clinical Importance. E. MARTIN SPENCER (ed.). Walter de Gruyter: 1983. Pp.664. ISBN 3-11-009562-9. DM 240.

Life in Ponds. By ALTHEA. Dinosaur Publications: 1984. Pp.24. Hbk ISBN 0-85122-412-1; pbk ISBN 0-85122-411-3. Hbk £2.95, pbk £1.10.

Macrophage-Mediated Antibody-Dependent Cellular Cytotoxicity. HILLEL S. KOREN (ed.). Dekker: 1983. Pp.361. ISBN 0-8247-7011-0. SwFr.150.

Mechanisms of DNA Replication and Recombination. UCLA Symposia on Molecular and Cellular Biology New Series, Vol. 10. NICHOLAS R. COZZARELLI (ed.). Alan R. Liss: 1983. Pp.876. ISBN 0-8451-2609-1. £104.

The Mind. By ANTHONY SMITH. Hodder & Stoughton: 1984. Pp.346. ISBN 0-340-26408-X. £10.95.

Modalités Rythmes et Mécanismes de L'Évolution Biologique: Gradualisme Phylétique ou Equilibres Ponctues? JEAN CHALINE (ed.). Editions du CNRS: 1983. Pp.335. ISBN 2-222-03259-8. Np.

Molecular Genetics of the Bacteria-Plant Interaction. A. PÜHLER (ed.). Springer-Verlag: 1983. Pp.393. ISBN 3-540-12798-4/0-387-12798-4. DM114, \$44.30.

Neurofilaments. CHARLES A. MAROTTA (ed.). University of Minnesota Press: 1983. Pp.238. ISBN 0-8166-1254-4. Np.

Of Mice, Models and Men: A Critical Evaluation of Animal Research. By ANDREW N. ROWAN. State University of New York Press: 1984. Pp.323. Hbk ISBN 0-87395-776-8; pbk 0-87395-777-6. Hbk \$34.50, pbk \$12.95.

The Parvoviruses. KENNETH I. BERNIS (ed.). Plenum: 1984. Pp.410. ISBN 0-306-41412-0. £59.50.

Periphyton of Freshwater Ecosystems: Developments in Hydrobiology 17. ROBERT G. WETZEL (ed.). Dr. W. Junk: 1983. Pp.346. ISBN 90-6193-768-X. Dfl.235, \$87.50.

The Pharmacology of Nerve and Muscle in Tissue Culture. By ALAN L. HARVEY. Croom Helm: 1984. Pp.252. ISBN 0-7099-1228-5. £19.95.

Photogenerated Reagents in Biochemistry and Molecular Biology. By H. BAYLEY. Elsevier: 1983. Pp.187. Hbk ISBN 0-444-80530-3; pbk ISBN 0-44480520-6. Pbk Dfl.58, \$22.50.

Physiological Ecology of Plants of the Wet Tropics. Tasks for Vegetation Science, Vol.12. E. MEDINA et al. (eds). Dr W. Junk: 1984. Pp.254. ISBN 90-6193-952-6. Dfl.160, \$60, £40.

Plant Molecular Biology, Vol.12: UCLA Symposia on Molecular and Cellular Biology New Series. ROBERT B. GOLDBERG (ed.). Alan R. Liss: 1983. Pp.498. ISBN 0-8451-2611-3. £60.

Practical Biology: A Guide to Teacher Assessment. By M.K. SANDS and P.E. BISHOP. Bell & Hyman: 1984. Pp.124. Pbk ISBN 0-7135-1405-1. £4.95.

Principles of Nucleic Acid Structure. By WOLFRAM SAENGER. Springer-Verlag: 1983. Pp.556. Hbk ISBN 3-540-90762-9/0-387-90762-9; pbk ISBN 3-540-90761-0/0-387-90761-0. Pbk DM79, \$29.50.

Progress in Botany: Morphology, Physiology, Genetics, Taxonomy, Geobotany. KARL ESSER et al. (eds). Springer-Verlag: 1983. Pp.404. ISBN 3-540-12997-9/0-387-12997-9. DM178, \$69.10.

Progress in Molecular and Subcellular Biology. Vol.8. F.E. HAHN (eds). Springer-Verlag: 1983. Pp.147. ISBN 3-540-12590-6/0-387-12590-6. DM89, \$34.60.

Radiation-Induced Chromosome Damage in Man. Progress and Topics in Cytogenetics, Vol.4. TAKAOKI ISHIHARA and MASAO S. SASAKI (eds). Alan R. Liss: 1984. Pp.650. ISBN 0-8451-2404-8. £75.

Sensory Experience, Adaptation, and Perception: Festschrift for Ivo Kohler. LOTHAR SPILLMANN and BILL R. WOOTEN (eds). Lawrence Erlbaum: 1984. Pp.748. ISBN 0-898-59218-6. £55.

The Smithsonian Institution 2nd Edn. By PAUL H. OEHSE. Bowker: 1983. Pp.223. ISBN 0-86531-300-8. £21.75.

So Excellent a Fish: The Classic Study of the Lives of Sea Turtles (Revised). By ARCHIE CARR. Charles Scribner: 1984. Pp.280. \$15.95.

Terpenoids and Steroids, Vol.12. J.R. HANSON et al. (eds). Royal Society of Chemistry: 1983. Pp.354. ISBN 0-85186-356-6. £56, \$100.

Tilapia Fishes of the General Sarotherodon, Oreochromis and Danakilia. By ETHELWYNN TREWAVAS. British Museum (Natural History): 1983. Pp.583. ISBN 0-565-00878-1. £50.

Treatment of Hemifacial Microsomia. EGIL P. HARVOLD (ed.). Alan R. Liss: 1983. Pp.247. ISBN 0-8451-0229-X. £37.

Applied Biological Sciences

Methods in Genetic Epidemiology. Contributions to Epidemiology and Biostatistics, Vol.4. By N.E. MORTON, D.C. RAO and J.-C. LALOUE. Karger: 1983. Pp.262. ISBN 3-8055-3668-2. SwFr. 164, DM 196, \$98.25.

Modulation and Mediation of Cancer by Vitamins. F.L. MEYSKENS and K.N. PRASAD (eds). Karger: 1983. Pp.350. ISBN 3-8055-3526-0. SwFr. 165, DM 198, \$99.

The Nature and Practice of Biological Control of Plant Pathogens. By R.J. COOK and K.F. BAKER. American Phytopathological Society: 1983. Pp.539. ISBN 0-89054-053-5. \$38 (members), \$43 (non-members).

Neurobehavioral Methods in Occupational Health. R. GILIOLI, M.G. CASSITTO and V. FOA (eds). Pergamon: 1983. Pp.314. ISBN 0-08-029802-8. £39, \$70.

New Anticancer Drugs: Mitoxantrone and Bisantrene. Monograph Series of the European Organization for Research on Treatment of Cancer, Vol.12. M. ROZENCWEIG, D.D. VON HOFF and M.J. STAQUET (eds). Raven: 1983. Pp.210. ISBN 0-89004-888-6. \$31.

Ovulation: Methods for its Prediction and Detection. Current Topics in Reproductive Endocrinology, Vol.3. S.L. JEFFCOATE (ed.). Wiley: 1983. Pp.126. ISBN 0-471-90196-2. £12.50, \$23.50.

Pain and Its Management. International Encyclopedia of Pharmacology and Therapeutics, Section 112. N.E. WILLIAMS and H. WILSON (eds). Pergamon: 1983. Pp.110. ISBN 0-08-029822-2. £25, \$45.

Pain Measurement and Assessment. R. MELZACK (ed.). Raven: 1983. Pp.309. ISBN 0-89004-893-2. \$40.

Pentoses and Lignin. Advances in Biochemical Engineering/Biotechnology, Vol.27. Springer-Verlag: 1983. Pp.186. ISBN 3-540-12182-X/0-387-12182-X. DM 86, \$34.20.

Pharmaceuticals and Health in the Third World. S.J. PATEL (ed.). Pergamon: 1983. Pp.331. ISBN 0-08-030210-6. £10.50, \$21.

Psychology

Behavioral Assessment and Rehabilitation of the Traumatically Brain-Damaged. Applied Clinical Psychology. BARRY A. EDELSTEIN and EUGENE T. COUTURE (eds). Plenum: 1984. Pp.346. ISBN 0-306-41295-0. \$39.

Cognitive Behavior Therapy with Children. Applied Clinical Psychology. ANDREW W. MEYERS and W. EDWARD CRAIGHEAD (eds). Plenum: 1984. Pp.493. ISBN 0-306-41291-8. £47.50.

Comprehensive Handbook of Psychopathology. HENRY E. ADAMS and PATRICIA B. SUTKER (eds). Plenum: 1984. Pp.1091. ISBN 0-306-41222-5. Np.

Fundamentals of Neuropsychopharmacology. By ROBERT S. FELDMAN and LINDA F. QUENZER. Sinauer: 1984. Pp.508. ISBN 0-87893-178-3. £29.

Language and Cognition: Essays in Honor of Arthur J. Bronstein. Cognition and Language. LAWRENCE J. RAPHAEL et al. (eds). Plenum: 1984. ISBN 0-306-41433-3. Np.

Neuroethology and Behavioral Physiology: Roots

and Growing Points. FRANZ HUBER and HUBERT MARKL (eds). Springer-Verlag: 1983. Pp.412. ISBN 3-540-12644-9/0-387-12644. DM80, \$31.10.

Psychology in Science: Towards a Universal Science of Human Progress. By KEVIN R.D. SHEPHERD. Rose-King Publications, PO Box 13, Cambridge CB4 1EZ, England: 1984. Pp.205. ISBN 0-9508690-0-0. £9.75.

General

Against Destruction. By DAWSON JACKSON. Lillstock Press, 5A Wellington Place, London NW8 7PB, England: 1984. Pp.356. Pbk ISBN 0-85635-535-6. Pbk £4.95.

Alcoholism in the Elderly: Social and Biomedical Issues. JAMES T. HARTFORD and T. SAMORAJSKI (eds). Raven: 1984. Pp.288. ISBN 0-89004-924-6. \$61.

Analyzing Activity Areas: An Ethnoarchaeological Study of the Use of Space. By SUSAN KENT. University of New Mexico Press: 1984. Pp.259. Hbk ISBN 0-8263-0718-3; pbk ISBN 0-8263-0719-1. Hbk \$24.95; pbk \$12.95.

Assessment and Programming for Young Children with Low-Incidence Handicaps. CECIL R. REYNOLDS and JULIA H. CLARK (eds). Plenum: 1983. Pp.347. ISBN 0-306-41469-4. £42.50.

Basics: SPSS-X. SPSS Inc./McGRAW-HILL: 1984. Pp.214. ISBN 0-07-060524-6. £10.50.

Biomass, Catalysts and Liquid Fuels. By I.M. CAMPBELL. Holt, Rinehart & Winston: 1983. Pp.169. ISBN 0-03-910464-8. £9.50.

Bone and Mineral Research, Annual 2. A Yearly Survey of Developments in the Field of Bone and Mineral Metabolism. WILLIAM A. PECK (ed.). Elsevier: 1984. Pp.432. ISBN 0-444-90337-2. Dfl. 190, \$73.

The Chief: Doctor William Osler. By R. PALMER HOWARD. Science History Publications: 1983. Pp.194. ISBN 0-88135-000-1. \$20.

Child Nurturance, Vol. 4: Child Nurturing in the 1980s. ROBERT P. BOGER et al. (eds). Plenum Press: 1984. Pp.194. ISBN 0-306-41505-4. Np.

Collected Works of Meghnad Saha. SANTIMAY CHATTERJEE (ed.). Orient Longman: 1982. Pp.591. ISBN 86131-348-8. Rs.75.

A Comprehensive Guide to the Therapeutic Use of Trascior. J. STEVO (ed.). Karger: 1984. Pp.115. ISBN 3-8055-3836-7. SwFr. 38, DM45, \$22.75.

Compulsive Gamblers. BY MARK G. DICKERSON. Longman: 1984. Pp.156. Pbk ISBN 0-582-29606-4. £3.25.

Computational Methods in Bifurcation Theory and Dissipative Structures. By M. KUBICEK and M. MAREK. Springer-Verlag: 1983. Pp.243. ISBN 3-540-12070-X. DM108, \$41.90.

Creativity as an Exact Science: The Theory of the Solution of Inventive Problems. Studies in Cybernetics 5. By G.S. ALTSHULLER. Gordon and Breach: 1984. Pp.319. ISBN 0-677-21230-5. \$54.

Dynamic Aspects of Language Processing: Focus and Presupposition. By J. ENGELKAMP and H.D. ZIMMER. Springer-Verlag: 1983. Pp.102. ISBN 3-540-12433-0/0-387-12433-0. DM 59, \$22.90.

Encyclopedia of Composite Materials and Components. MARTIN GRAYSON (ed.). Wiley: 1983. Pp.1161. ISBN 0-471-87357-8. £94.50.

Environmental Philosophy: A Collection of Readings. R. ELLIOT and A. GARE (eds). The Open University: 1983. Pp. 303. Pbk ISBN 0-335-10407-X. £8.95.

The Finance of Cities in West Germany: Vol. 21, Part 1 Progress in Planning. By R.J. BENNETT. Pergamon Press: 1983. Pp.62. ISBN 0-08-031462-7. £11, \$20.

Five Hundred Points of Good Husbandry. By THOMAS TUSSER. Oxford University Press: 1984. Pp.344. ISBN 0-19-286040-2. Pbk £3.50.

The Granite Garden: Urban Nature and Human Design. By A. WHISTON SPIRN. Basic Books: 1984. Pp. 334. ISBN 0-465-02699-0. \$25.95.

Health and Ways of Living: The Alameda County Study. By LISA F. BERKMAN and LESTER BRESLOW. Oxford University Press: 1983. Pp.237. ISBN 0-19-503216-0. £24.

Industry and the Environment in Perspective. The Proceedings of a Symposium Organised by The

Industrial Division of The Royal Society of Chemistry as Part of the Annual Chemical Congress, 1983. R.E. HESTER (ed.). Royal Society of Chemistry: 1983. Pp.222. ISBN 0-85186-895-9. £13, \$24. Discount for Members.

Information Sources in the Medical Sciences, 3rd Edn. L.T. MORTON and S. GODBOLT (eds.). Butterworths: 1984. Pp.534. ISBN 0-408-11473-8. £38.

The Jahn-Teller Effect: A Bibliographic Review. By I.B. BERSUKER. Plenum Press: 1984. Pp.589. ISBN 0-306-65206-4. Np.

The Legal Regime of Fisheries in the Caribbean Region. Lecture Notes on Coastal and Estuarine Studies, Vol. 7. By W.R. EDESON and J.F. PULVENIS. Springer-Verlag: 1983. Pp.204. ISBN 3-540-12698-8/0-387-12698-8. DM 43, \$16.70.

Lehre und Forschung: Autobiographische Erinnerungen. By ALBERT FREY-WYSSLING. Wissenschaftliche Verlagsgesellschaft mbH Stuttgart: 1984. Pp.192. ISBN 3-8047-0695-9. DM 39.

Local Plans in British Land Use Planning. By P. HEALEY. Pergamon: 1983. Pp.324. ISBN 0-08-025242-7. £16.50, \$30.

Models of Reality: Shaping Thought and Action. JACQUES RICHARDSON (ed.). Lomond Publications: 1984. Pp.328. ISBN 0-912338-35-0. Np.

Natural Systems for Development: What Planners Need to Know. RICHARD A. CARPENTER (ed.). Macmillan: 1983. Pp.485. ISBN 0-02-949290-4. \$37.50.

No Clear Reason: Nuclear Power Politics. Radical Science 14. Edited by the Radical Science Collective. Free Association Books, 26 Freegrove Road, London N7 9RQ, England: 1984. Pp.182. ISBN 0-946960-00-3. £5, \$8.

Noise-Induced Transitions: Theory and Applications in Physics, Chemistry and Biology. By W. HORSTHEMKE and R. LEFEVER. Springer-Verlag: 1984. Pp.318. ISBN 3-540-11359-2/0-387-11359-2. DM 108, \$41.90.

Nouvelles Recherches dans le Domaine des Composés Macromoléculaires. By ELENA CEAUSCESCU. Pergamon: 1984. Pp.451. ISBN 0-08-030725-6. £45, \$72.

Nuclear Arms Control: With Effective International Agreements. By J. DAHLITZ. George Allen & Unwin: 1983. Pp.238. Hbk ISBN 0-04-341023-5; pbk ISBN 0-04-341024-3. Hbk £15, \$25; pbk £4.95, \$8.95.

On Size and Life. By THOMAS A. McMAHON and JOHN TYLER BONNER. W. H. Freeman: 1984. Pp.255. ISBN 0-7167-5000-7. £14.50.

Ponds and Pools — Oases in the Landscape. By KLAUS KABISCH and JOACHIM HEMMERLING. Croom Helm: 1984. Pp.259. 0-7099-1545-4. £8.95.

Prevention of Alcohol Abuse. PETER M. MILLER and TED D. NIRENBERG (eds). Plenum: 1984. Pp.520. ISBN 0-306-41328-0. Np.

Realm of the Long Eyes: A Brief History of Kitt Peak National Observatory. By JAMES E. KLOEPPEL. Univelt Incorporated, PO Box 28130, San Diego, California 92128: 1983. Pp.136. ISBN 0-912183-01-2. \$15.

Reliability Evaluation of Power Systems. By ROY BILLINTON and RONALD N. ALLAN. Plenum: 1984. Pp.432. ISBN 0-306-41450-3. Np.

Reorienting Health Services: Application of a Systems Approach. CHARLES O. PANNENBORG et al. (eds). Plenum: 1984. Pp.374. ISBN 0-306-41481-3. Np.

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Science and Parascience: A History of the Paranormal 1914-1939. By BRIAN INGLIS. Hodder & Stoughton: 1984. Pp.382. ISBN 0-340-26325-3. £12.95.

Science and Technology in Medieval India: A Bibliography of Source Materials in Sanskrit, Arabic and Persian. By A. RAHMAN. Indian National Science Academy, New Delhi: 1982. Pp.719. \$70.

The Scientific Basis of Flotation. KENNETH J. IVES (ed.). Martinus Nijhoff: 1984. Pp.429. ISBN 90-247-2907-6. Dfl. 150, \$56, £37.50.

Semmelweis' Krankheit. By ISTVAN BENEDEK. Akadémiai Kiadó, Budapest: 1983. Pp.110. ISBN 963-05-3428-2. Np.

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One of fifteen new products this week is a system based on Edman degradation which will detect amino acids at 1 pmol. And NCI announces the availability of serum components.

● Two new nonisotopic assays for monitoring thyroid function have been announced by Syva, the Syva Advance thyroxine assay and the Syva Advance T-3 uptake assay. Using fluorescence energy transfer immunoassay (FETI), the assays are designed to run on the Syva Advance system. The thyroxine assay measures total (protein-bound and unbound) thyroxine in serum. The T-3 uptake assay measures human-serum binding capacity for thyroid hormone. The assays require only a small sample size (25 μ l for thyroxine; 40 μ l for T-3 uptake), and both assays use a simple two-point calibration.

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● The Gilson 401 dilutor is a single-syringe diluter that dispenses, pipettes and dilutes with very high accuracy and precision for liquid sample handling. In addition to the usual performances expected from an automatic and programmable dilutor, the Gilson 401 will perform sequential dilutions, aspirate up to 12 different samples in a single cycle, split an aspirated volume of reagent into up to 12 fractions and function in "reverse pipetting" mode to eliminate the risk of cross contamination. Programming is via a simple keypad controller and its microprocessor based circuitry can store up to 90 programs for easy access. This dilutor may also be externally controlled from a computer.

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● A comprehensive reference list is available from Vector Laboratories covering 120 publications using the ABC immunoperoxidase staining system. In addition to light microscopy with polyclonal antibodies on paraffin sections, applications include staining of frozen sections, cytocentrifuge preparations, solid phase enzyme immunoassays, nitrocellulose blots, and pre- and post-embedding EM using monoclonal antibodies, lectins and biotinylated polynucleotide hybridizing probes.

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● The derivatizing reagent phenylisothiocyanate (PITC), the major reactant in the Edman degradation procedure for sequencing proteins and peptides, has been modified by Waters Associates to provide a new HPLC technique for amino acid analysis. Termed PICO-TAG, the new method offers 1 picomole detection in analysis of primary and secondary amino acids in only 15 minutes.

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These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.



The new pH/mV calibrator from Chemtrix.

● An extensive range of avidin conjugates for use in indirect immunoassays is available from Tago Inc. The avidin conjugates are now available with FITC, rhodamine, Texas Red, horseradish peroxidase, alkaline phosphatase, or beta-galactosidase. Quality performance is confirmed by ELISA testing and histological microscopy. These new avidin conjugates are companion products to a wide line of biotinylated xenogenic affinity-isolated antibodies and F(ab')₂ fragments specific for human, mouse, rat, rabbit or pig Ig.

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● The new Kratos MS 25 RFA mass spectrometer features new magnet technology, faster scanning capabilities and a higher mass range than previous models. The fast scanning (0.1s) magnet permits acquisition of high quality spectra from extremely narrow GC peaks, and the higher mass range of >3,000 AMU at 1 kV delivers high quality high mass spectra when used in conjunction with a fast atom bombardment source. Combined with the new DS 90 colour graphics data system, the MS 25 RFA offers levels of performance, previous available only using more expensive high resolution GC/MS systems.

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● The Chemtrix "pHault Finder" is a hand-held pH checker which can quickly and accurately test the calibration, temperature compensating circuits, input impedance and millivolt output of a pH meter. This unit stimulates the input from a pH electrode in pH units, 0 to 14 pH or mV units, +355 to -414 mV units. For each unit change in pH, the millivolt reading shifts ± 59.1 mV units. Additionally, the automatic and manual temperature compensating circuits of a meter may be verified with the pHault Finder in 25°C steps between 0° and 100°C.

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● The VG Instruments PlasmaQuad is a powerful new system for elemental analysis combining an inductively coupled plasma with a high-resolution quadrupole mass spectrometer (ICP-MS). The ICP-MS technique gives cleaner spectra than other analytical methods for lower limits of detection and low noise levels.

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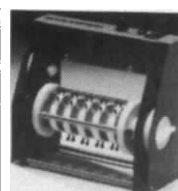
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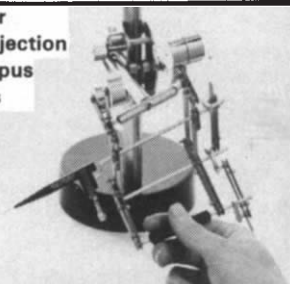
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ONE-STEP PROCEDURE PURIFIES



MONOCLONALS

Bio-Rad's DEAE Affi-Gel® Blue has been used successfully to purify rabbit and human IgG from whole serum. Now it's been reported¹ that this same one-step chromatographic procedure can be used to purify mouse monoclonal antibodies directly from ascites fluid under mild conditions—pH 7.2. The resulting IgG fraction is free of protease and nuclease activity and is stable to long term storage. The authors include a chromatogram (Fig. 1) showing the separation of IgG₁. The procedure is also effective for IgG₂.

1. Bruck, C., Portetelle, D., Gilneir, C. and Bollar, A., *J. Immunol. Methods*, 53, 313 (1982).

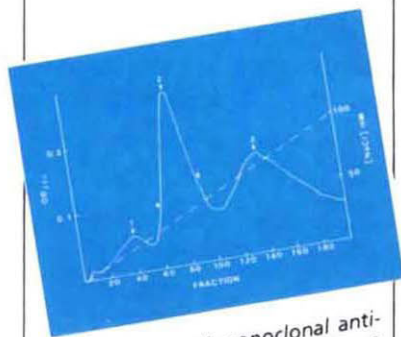


Fig. 1 Elution of monoclonal anti-urokinase antibodies (IgG₁) from a DEAE Affi-Gel Blue column.

See us at The American Society of Biological Chemists/AAI Booth S-3

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● The new brain slice chamber from Medical Systems Corporation of Great Neck, New York features a single base unit and two interchangeable top units. The Haas top configuration utilizes semi-submersion techniques, providing good stability for intracellular recording and rapid exchange for perfusion fluids. The Zbicz top is designed to maintain viable slices of tissue submerged under a constant flow of perfusing liquid. A range of techniques can be used for evaluation of drug actions. Cell longevity of 10 or more hours is possible. A companion temperature controller is offered which functions with either top chamber and regulates temperature $30^{\circ}\text{--}40^{\circ} \pm 0.2^{\circ}\text{C}$.

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● Dynatech Laboratories' AM572A multi-purpose Autowash washer/aspirator can deliver wash fluid and aspirate a complete 96-well MicroElisa plate in 60–90 seconds, depending on the specific test requirements. The machine is therefore suitable for the smaller laboratory with a limited through-put of Miro-Elisa tests as well as being capable of handling the demands of the large-scale user. It can also be used in certain RIA and blood-transfusion applications. A simple but accurate depth adjustment on the wash/aspirate manifold makes the AM572A Autowash particularly suited to working with tissue culture monolayers.

Circle No. 109 on Reader Service Card.

● A new test kit for the determination of angiotensin converting enzyme (ACE) in serum is available from Boehringer Mannheim. The colorimetric assay is based on the ability of ACE to convert hippury-glycyl-glycine into glycyl-glycine which is converted to trinitrophenyl-glycyl-glycine by trinitrobenzylsulphonate. The absorbance of trinitrophenyl-glycyl-glycine is measured at 420 nm. The test requires a sample of only 10 μl .

Circle No. 110 on Reader Service Card.

● Very low levels of NADH and NADPH can be detected using the new LKB bioluminescent assay. The reagents use bacterial luciferase coupled to a specific oxidoreductase enzyme. There are therefore two reagents, one for NADH, the other for NADPH. These reagents give a vastly improved (1,000-fold) sensitivity compared with spectrophotometric assay for NADH, NADPH and associated enzyme assays. An application paper is available from LKB.

Circle No. 111 on Reader Service Card.

● New from Cruachem, ColorTAG monomers can be used during the synthesis of a mixed DNA probe to determine the success or failure of a mixed addition. The deprotection effluent from each of the monomers is a distinctive colour and so the actual nucleotide ratio at a degenerate site can be determined by visible spectroscopy. This provides confirmation that all possible sequences are indeed present in a mixed probe.

Circle No. 112 on Reader Service Card.

● For decontamination of laboratory glassware used with radioactive solutions, ICN Radiochemicals is marketing Dekasol. After a 24-hour soak at room temperature in 5% Dekasol and distilled water, residual activity for carbon-14, tritium and phosphorus-32 is usually 0.2% of the original activity. For more stubborn radioactive substrates, such as those containing iron-49 and iodine-131, soaking in a 20% solution of Dekasol at 50°C for 2 hours provides effective decontamination.

Circle No. 113 on Reader Service Card.

● Zetabind is a charge-modified membrane matrix for DNA, RNA and protein transfer applications. Manufactured by AMF Cuno, Zetabind is composed of charge modified nylon 66 which results in an electrostatic adsorption of anionic macromolecules. Binding capacities of 480 $\mu\text{g g}^{-1}$ have been reported and this highly efficient binding permits re-use of transfers for re-probing. Zetabind, unlike DBM and DPT papers, does not require chemical activation and can be stored indefinitely at room temperature. Efficient binding of anionic macromolecules in low salt buffers permits electrophoretic transfers from gels. It also provides enhanced binding for capillary blots.

Circle No. 114 on Reader Service Card.

Frozen serum panels from NCI

A VARIETY of serum components (including peptide hormones, viral antigens, isoenzymes, glycoproteins, antibodies, immune complexes, tumour-associated antigens, carbohydrates, phospholipids and nucleotides) have been reported to be useful in cancer diagnosis and/or in monitoring cancer treatment or recurrence. The US National Cancer Institute is interested in evaluating serum assays that are potentially useful in the diagnosis of cancer. Coded panels composed of 1 ml aliquots of pretreatment frozen sera from patients with various neoplasms, from benign disease patients and from healthy controls are available to investigators to evaluate assays in which preliminary results indicate the ability to discriminate between cancer patients and controls. Promising results may form the basis for a subsequent grant application. Preliminary data documenting a useful test must be submitted and should include: a brief description of the assay, results of patients with cancer, results of patients with non-malignant disease, results in healthy control subjects and reprints of published work, if available. Request for a coded serum panel should be sent to: Diagnosis Serum Panels, Project Officer NCI-Serum Bank, Diagnosis Branch, Westwood Bldg., Room 10A10, 5333 Westbard Avenue, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205. □